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Ricerche su sostanze ad azione antidotica prodotte da Miceti (Attività dell' *Aspergillus fumigatus*)

DR. ANNA MARIA VILLA
(*Centro di Micologia Umana e Comparata di Milano - Pavia*)

(24.1.1949)

Un numero notevole di ricerche si é accumulato negli ultimi anni intorno agli antibiotici. Di acquisizione più recente e assai meno noto é il fatto che molti funghi e batteri producono non solo sostanze antibiotiche, quindi attive direttamente sui germi, ma anche sostanze antidotiche, cioé sostanze che inattivano i prodotti tossici del metabolismo dei germi.

Fù NETER che nel 1945 potè osservare che la clavacina (sostanza prodotta dal *P. patulum* e come fu più tardi scoperto anche da numerosi altri aspergilli e penicilli: *P. claviforme*, *P. expansum*, *Asp. clavatus*, *Asp. giganteus*) e la piocianasi diminuivano la tossicità della tossina tetanica mentre la penicillina, la streptomicina e l'actinomicina A erano prive di questra azione.

In seguito nel 1945 G. RAMON, R. RICHOU, P. RAMON studiarono l'azione del filtrato di *B. subtilis* sulle tossine difterica, tetanica, stafilococcica: azione che si manifesta con una diminuzione di tossicità delle dette tossine quando siano poste in contatto con il filtrato.

Gli autori hanno chiamato *proprietà antidotica* la proprietà dei filtrati di distruggere le tossine microbiche.

Nel corso di queste esperienze gli autori hanno confermato le asserzioni di NETER secondo le quali la penicillina purificata, come é preparata per uso terapeutico, mentre é dotata di notevole potere antibiotico, é completamente priva di potere antidotico. Conferme a questa affermazione si trovano in lavori americani. Per questo gli Autori ebbero l'idea di ricercare se i filtrati di *P. notatum* fossero dotati di potere antidotico che non si troverebbe nella penicillina perché eliminato nei processi di estrazione e di purificazione.

La scoperta del potere antidotico nel filtrato di *P. notatum* fece supporre che la medesima proprietà fosse posseduta da altri germi antagonisti.

Nel 1947 RAMON, LEVADITI, RICHOU, dimostrarono a loro volta che i filtrati di *Actinomyces* sono dotati di potere antidotico verso la tossina stafilococcica e difterica mentre la streptomicina purificata é priva di questo potere.

Molto recentemente CAVALLI poté confermare in un suo lavoro le affermazioni degli Autori precedenti. Egli provó sei ceppi diversi di Penicilli, 5 dei quali si dimostrarono dotati di potere antidotico mentre uno ne era privo. Tutti i detti ceppi erano anche dotati di potere antibiotico.

Gli ultimi lavori di RAMON e RICHOU studiano l'effetto del siero sul potere antidotico e indicano un metodo di dosaggio del principio antidotico; usando dosi scalari di filtrato su dosi fisse di tossina per un tempo determinato, definiscono la unità antidotica, come la più piccola quantità di filtrato che

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[1]) Ringrazio il Dott. Cavalli e l'Istituto Sieroterapico Milanese per l'aiuto ed i mezzi fornitimi durante lo svolgimento delle ricerche.

distrugge totalmente in 6 ore a 37 gradi 100 dosi minime emolitiche di tossina stafilococcia.

Concludendo: dall'esame dei lavori fino ad oggi compiuti sul potere antidotico si può affermare che sono stati studiati i filtrati di *P. notatum*, *Act. griseus*, *B. subtilis*, inoltre due antibiotici purificati; la clavacina e la piocianasi. Queste sostanze sono attive sulle tossine stafilococcica, difterica e tetanica. Dei filtrati i primi due sono termolabili, l'ultimo é termostabile.

Scopi della presente ricerca

Nelle presenti ricerche si é voluto chiarire alcuni punti rimasti oscuri nei lavori precedenti, ed estendere i risultati. Si é proceduto quindi innanzitutto alla ricerca di ceppi attivi non solo tra i miceti già studiati ma anche tra gli aspergilli ed i lieviti, studiando contemporaneamente l'attività antidotica e l'attività antibiotica di questi miceti. Si é poi messo a punto un metodo di titolazione (quello proposto da Richou fu pubblicato mentre queste ricerche erano già in corso), che comporta alcune differenze rispetto al metodo di Richou, e si é dimostrato più pratico del medesimo. Invece che 100 dosi minime emolitiche, la quantità di tossina é, come vedremo meglio in seguito, 2,5 dosi minime emolitiche da inattivare in un tempo breve (10'); di conseguenza si può ridurre il tempo di contatto fra filtrato e tossina a mezz'ora a 37 gradi.

Mediante questo metodo di titolazione é stato possibile studiare i seguenti problemi:

1. influenza di diversi terreni sulla produzione del principio antidotico;
2. andamento del titolo del principio antidotico nel tempo (curva di produzione);
3. influenza del pH di partenza e del pH che successivamente si stabilisce nel terreno culturale;
4. stabilità nel tempo a temperatura ambiente;
5. resistenza al calore;
6. resistenza a vari pH, come introduzione a una ricerca sulla purificazione ed isolamento della sostanza;
7. dializzabilità.

Si é quindi cercato di chiarire il modo di azione del principio antidotico sulle tossine. Ramon e Richou paragonano l'azione del principio antidotico a quella del formolo, ma non hanno nessuna base per affermarlo: per poterlo dire occorrerebbe sapere se viene distrutto solo il potere tossico e non il potere antigene delle tossine. Ci é sembrato interessante raccogliere informazioni su questo punto per vedere di chiarire come agisca il potere antidotico sulle tossine. Ho quindi studiato l'azione del principio antidotico contemporaneamente sul potere antigene e su quello tossico della tossina difterica raggiungendo alcune conclusioni che saranno esposte in seguito.

Tecnica del saggio del potere antidotico sulla tossina stafilococcica.

Per dimostrare la presenza del potere antidotico nei numerosi filtrati studiati nella presente ricerca, mi sono servita della tossina stafilococcica, in quanto la medesima offre un semplice metodo di titolazione in vitro basato sulla proprietá della tossina stessa di emolisare i globuli rossi di coniglio.

Si pongono in provette quantità scalari della sostanza da esaminare e, praticamente si parte da valori di 0,4 cc., non considerandosi quantità superiori, si scende a 0,2 cc. della sostanza pura, poi si diluisce 1 : 10 e si pongono quantità decrescenti da 1 cc. a 0,2 cc. — Si aggiunge 0,2 cc. di tossina stafilococcica titolante 250 DMH per cc. diluita 1 : 20 in modo da avere in ogni provetta 2,5 DMH. Si porta al volume di 1,8 cc. con soluzione fisiologica, e si pongono le provette a bagno maria a 37° per 30 minuti. Dopo 30 minuti si aggiunge in ogni provetta 0,2 cc. di una sospensione di globuli rossi di coniglio centrifugati e lavati 3 volte, diluiti al 50 % con soluzione fisiologica (in modo da usare globuli rossi al 5 %). Si lasciano le provette a bagno maria per 10 minuti e poi si centrifuga per 1 minuto. Si fa un controllo ponendo a contatto la tossina con i globuli rossi per assicurarsi della attività della tossina stessa.

Basandosi sul principio che la tossina stafilococcica determina la emolisi dei globuli rossi di coniglio, con questo metodo si può immediatamente constatare l'attività delle sostanze in esame: se le sostanze sono fornite di potere antidotico, neutralizzano la tossina e non compare emolisi, se ne sono prive, la tossina determinerà l'emolisi dei globuli rossi. Se la sostanza neutralizza la tossina solo in parte, avremo una percentuale di emolisi che sarà in funzione della quantità di tossina residua.

Per valutare il potere antidotico si é preso il valore della diluizione massima di sostanza che inattiva totalmente la tossina stafilococcica senza tener conto delle inattivazioni parziali; questo valore dà il titolo in unità antidotiche (U.A.). Confrontando questo metodo con il metodo di Ramon e Richou, si può stabilire con un semplice calcolo che l'unità antidotica di Ramon e Richou corrisponde a 3 unità antidotiche secondo il mio metodo.

Il potere antidotico di miceti e di lieviti

I vari ceppi esaminati provengono dalla collezione dell' Istituto di Anatomia Patologica dell' Università di Milano o sono stati isolati recentemente in natura. In ogni caso si é provveduto alla coltivazione dei miceti su un terreno all'agar carota, dei lieviti su un terreno all'agar glucosio, tenendo in termostato a 25° per 3 giorni. Dalla cultura su terreno solido di 3 giorni si é partiti per la semina delle spore su terreno di Abraham con l'aggiunta di peptone all' 1 %, terreno che si é dimostrato più favorevole per il rapido accrescimento dei ceppi. La semina delle spore viene fatta con una normale ansa di platino e le culture vengono tenute in termostato a 25°. Su questo terreno i miceti crescono rapidamente, formando un feltro omogeneo in superficie, mentre la sostanza attiva si va accumulando nel sottostante liquido culturale. Dopo esperimenti preliminari di orientamento si é visto che la massima resa di principio antidotico si aveva fra la settima e la decima giornata: quindi le titolazioni venivano eseguite nei giorni suddetti, tenendo però in ogni caso conto del grado di sviluppo raggiunto dal micete.

Si é visto infatti che la migliore produzione di principio antidotico si ha quando il micelio raggiunge uno sviluppo sufficientemente rigoglioso nei primi giorni di accrescimento, e soprattutto quando la cultura ha scarsa tendenza a sporificare.

Il liquido culturale contenente la sostanza attiva veniva prelevato sterilmente e filtrato su comune carta da filtro. In ogni caso si é determinato il pH del filtrato stesso mediante indicatore Merk, salvo in casi particolari che verrano segnalati, in cui si é usato il metodo elettrometrico, per avere valori più esatti. Per ogni filtrato é stato sempre usato il metodo di titolazione descritto prima, servendosi per tutto il corso dell' esperimento della medesima partita di tossina stafilococcica.

Faccio notare a questo punto che in esperimenti successivi atti a dimostrare la produzione di principio antidotico nel tempo, si é visto che la quantità di sostanza raggiunge un massimo di concentrazione in un giorno determinato, mentre nei giorni precedenti e seguenti a questo massimo, si hanno quantità progressivamente decrescenti. Per avere il lavore esatto della quantità massima di principio antidotico prodotto dai vari miceti bisognerebbe determinare la curva di produzione per ogni ceppo. In queste richerche eseguite su un notevole numero di ceppi invece si é fatta una o due titolazioni intorno alla decima giornata. Di conseguenza i risultati esposti, mentre sono sufficienti a rivelare i ceppi produttori di sostanza antidotica, danno un valore solo approssimato della potenzialità di produzione di principio antidotico. Di ogni filtrato che si dimostrava fornito di potere antidotico é stato studiato anche il potere antibiotico, servendosi del metodo dei cilindri di vetro su piastra.

Riporto l'elenco dei ceppi studiati:

A) **Penicilli**

P. chloroleucon
P. notatum NRRL 1950
P. notatum NRRL 1951
Penicillium n. 1.2.3.4.5.6.7.8.9.10.11.12.13.14.15.16.17.18.19.20.21.30.31.32.40.41.42.43.44.45.46.47. (tutti questi ceppi numerati sono stati isolati direttamente da vari terreni di giardini e campagne).

Si sono dimostrati attivi i sequenti ceppi:

P. chloroleucon *P. notatum* NRRL 1950 *P. notatum* NRRL 1951
P. n. 5. 13. 44.

Nella tabella seguente riporto il valore del pH del filtrato, determinato con indicatore Merk, il valore del potere antidotico in unità, secondo quanto definito precedentemente, e il valore del potere antibiotico espresso per i primi 3 Penicilli in unità Oxford (come da determinazioni eseguite all' I.S.M.S.B.), per gli altri in mm. di alone di inibizione secondo il metodo dei cilindri su agar:

	pH	Unità antidotiche	Attività antibiotica
P. chloroleucon	8	10	10 U. Oxford
P. NRRL 1950	8	10	40 U. Oxford
P. NRRL 1951	8	5	30 U. Oxford
P.n. 5	7	5	10 mm. alone inibizione
P.n. 13	7	10	18 mm. alone inibizione
P.n. 21	7	5	13 mm. alone inibizione
P.n. 44	8	5	29 mm. alone inibizione

I ceppi dotati di potere antidotico sono stati identificati dal Centraalbureau Voor Schimmelcultures di Baarn (Olanda); essi sono:

5 *Penicillium* meleagrinum Biourge
13 *Penicillium Sartoryi* Thom.
44 *Penicillium Meleagrinum* Biourge

B) **Aspergilli**

A. fumigatus *A. oryzae* *A. terreus* *A. albus* *A. flavus*
tutti dell' Istituto di Anatomia Patologica dell' Università.
N. 7193 (National Collection of Type Cultures M.R.C.)
N. 5606 (National Collection of Type Cultures M.R.C.)
N. 597 (National Collection of Type Cultures M.R.C.)
A. niger 237 (Ist. Pasteur)
A. nidulans (Graziosi Roma)
A. nidulans (da Carate colombiana)
A. oryzae 239 (Langeron 170)
A. niger 1015 (American type cultures collection)
A. niger 6276 (American type cultures collection)
A. terreus Thom (C.B.S. 1)
A. terreus Thom (C.B.S. 2)
A. Wenti Wehmer
A. terreus 10029 (American type cultures collection)
A. niger v. Thiegem (C.B.S.)
A. niger v. Thiegem mut. *Fusca* Blochw (C.B.S.)
A. niger v. Thiegem radium strain (C.B.S. 1)
A. niger v. Thiegem radium strain (C.B.S. 2)
A. Wenti Wehmer 2
A. terreus Thom 1960

Si sono dimostrati attivi i seguenti ceppi:
A. fumigatus (I.A.P.)
A. niger 237 (Ist. Pasteur)
A. niger 597 (American type cultures collection)
A. niger 6276 (American type cultures collection)
A. niger mut. *Fusca* Blochw
A. fumigatus Fres.

Riporto in tabella i risultati analogamente a quanto fatto più sopra per i ceppi di Penicilli:

	pH	Unità antidotiche	Attività antibiotica
A. fumigatus I.A.P.	6	50	nulla
A. niger 237	6	5	nulla
A. niger 6376	6	5	nulla
A niger m. F. Blochw	7	5	nulla
A. niger 597	7	5	nulla
A. fumigatus Fres.	7	20	18 mm. alone inibizione

C) **Lieviti**

Lievito di panetteria
1F *Saccharomyces Barlowiski* (Inst. Forlanini Roma)

130 *Mycotorula kefir* Langeron 513
103 *Oidium asteroides* (*Geotrichum Castellani* 1931)
36 Starkow
134 *Mycotorula tropicalis* Castellani 1931
164 *Pityrosporum Dowling* Langeron 373 (1931)

Si sono dimostrati attivi i seguenti ceppi:

1F *Saccharomyces Barlowiski*
103 *Oidium asteroides*
130 *Mycotorula kefir*
134 *Mycotorula tropicalis*

Riporto in tabella i risultati analogamente a quanto fatto sopra per i Penicilli e Aspergilli:

	pH	Attività antidotica	Attività antibiotica
Mycotorula kefir	6	10	nulla
103 *Oidium aster.*	6	10	nulla
Mycotorula tropic.	6	5	nulla
Saccaromyces Barlow	6	5	nulla

Sono stati studiati inoltre un ceppo di *Actinomyces griseus* che non ha mastrato nessuna attività antidotica e un ceppo di *B. subtilis*.

Questo ceppo é stato coltivato sui terreni seguenti:

Terreno di Sauton senza asparagina con Casidrol al 0,5 %

Terreno di Sauton senza asparagina con Endamina al 0,5 %

(Il Casidrol é un idrolizzato enzimatico di caseina e di carne in vendita in Inghilterra. L'Endamina é un idrolizzato di proteine animali dell' I.S.M.S.B.).

La cultura era tenuta in termostato a 37°. La titolazione della sostanza prodotta dal *B. subtilis* venne effettuata in quinta e decima giornata, previa centrifugazione del liquido culturale per eliminare i germi.

Riporto i risultati ottenuti nei due terreni:

Quinta giornata			Decima giornata	
	pH	Unità antidotiche	pH	Unità antidotiche
Sauton endamina	6,5	5	6,5	5
Sauton casidrol	6,5	5	6,5	10

Riassumendo, sono stati esaminati 68- ceppi diversi e precisamente:

34 ceppi di *Penicilli*; 25 ceppi di *Aspergilli*; 7 ceppi di *lieviti*; 1 ceppo di *Actinomyces*; 1 ceppo di *B. subtilis*;

Di questi hanno mostrato attività antidotica:

7 ceppi di *Penicilli*; 6 ceppi di *Aspergilli*; 4 ceppi di *lieviti*; 1 ceppo di *B. subtilis*.

La frequenza percentuale per i casi studiati é: del 20,6 % per i *Penicilli*, del 24 % per gli *Aspergilli* e del 57,1 % per i lieviti; dal cui esame risulta chiaramente la notevole diffusione in natura del principio antidotico.

Influenza delle condizioni di coltura sulla produzione di principio antidotico da parte dell' A. fumigatus

Fra i vari ceppi produttori di principio antidotico il ceppo che ha mostrato il massimo di attività é stato l'*A. fumigatus*.

Nella letteratura l'*A. fumigatus* é noto come produttore di sostanze antibiotiche che sono state studiate dettagliatamente da Waksman e Geiger. Queste sostanze sono state separate e purificate chimicamente: esse sono quattro e differiscono sia nella costituzione chimica che nello spettro antibiotico.

Esse sono: Spinulosina $C_8H_8O_5$; Fumigatina $C_8H_8O_4$; Fumigacina o acido elvolico $C_{33}H_{44}O_8$; Gliotossina $C_{13}H_{14}O_4N_2S_2$.

Mentre tutte le precedenti sostanze isolate da colture di *A. fumigatus* mostrano una attività antibiotica verso lo Stafilococco, il ceppo di *A. fumigatus* usato in questi esperimenti e che si é dimostrato fornito di notevole attività antidotica, in prove ripetute non ha mai mostrato nessuna attività antibiotica. Questo fatto può da solo escludere che il principio antidotico studiato corrisponda a una delle quattro sostanze già studiate e chimicamente note, prodotte dall'*A. fumigatus*.

Il ceppo di *A. fumigatus* é stato studiato dettagliatamente sia per quanto riguarda le condizioni di cultura sulla produzione del principio antidotico, sia per quanto riguarda alcuni caratteri del principio stesso.

Innanzi tutto si é proceduto alla coltivazione dell'*A. fumigatus* su vari terreni culturali per vedere l'influenza della composizione del terreno sulla produzione del principio antidotico.

Si sono preparati i seguenti 10 terreni:

1. Abraham semplice
2. Abraham peptone IAP 1 %
3. Abraham peptone Vismara 2 %
4. Abraham peptone Vismara 1 %
5. Abraham peptone Vismara 0,5 %
6. Abraham casidrol 1 %
7. Abraham casidrol 0,5 %
8. Abraham endamina 2 %
9. Abraham endamina 1 %
10. Abraham endamina 0,5 %

Il pH dei terreni dava un valore di circa 6 per tutti i terreni, con indicatore Merk.

In questo caso di é proceduto alla semina delle spore paertaendo da una cultura su agar carota dell'*A. fumigatus*, e facendo una sospensione delle spore stesse mediante aggiunta all'agar cultura di laurilsulfonato diluito 1 : 10.000 con acqua distillata. In ogni matraccio contenente 60 cc. di terreno liquido veniva aggiunto 0,5 cc. della sospensione di spore, in modo da avere in ogni matraccio una quantità grossolanamente uguale di spore. Le culture erano tenute in termostato a 27°. Sin dai primi giorni si é notato una notevole differenza di sviluppo del micete nei diversi terreni, avendosi uno sviluppo scarsissimo nel terreno 1, scarso nei terreni 2.3.4, rigoglioso senza sporificazione nei terreni 6.7, rigoglioso con sporificazione nei terreni 5.8.9.10. Faccio notare questo perché probabilmente sulla produzione della sostanza attiva influisce non solo la composizione del liquido culturale ma anche il grado di sviluppo raggiunto dal micete.

Si sono eseguite due titolazioni in quinta e in decima giornata, ottenendo i risultati che riporto in tabella, indicando i valori del pH raggiunto dal liquido:

	quinta giornata		decima giornata	
	pH	Unità antidotiche	pH	Unità antidotiche
1.	6	5	6	5
2.	6	< 5	6	$> 5 < 10$
3.	6	5	6	10
4.	6	10	6	10
5.	6	$> 5 < 10$	6	$> 10 < 20$
6.	6	67	7	67
7.	6	33	7	67
8.	6	< 5	7,5	5
9.	5	5	6,5	10
10.	6	< 5	6	10

Come si vede la massima resa si é evuta per i terreni: Abraham casidrol 1 % e 0,5 %, mentre il terreno Abraham peptone IAP che normalmente dà un titolo di circa 50 unità antidotiche; in questo caso ha dato una produzione scarsa di sole 10 unità.

Questo esperimento, mentre rivela la grande variabilità di sviluppo di uno stesso ceppo su vari terreni, conferma anche l'importanza del modo di accrescimento sulla produzione del principio attivo.

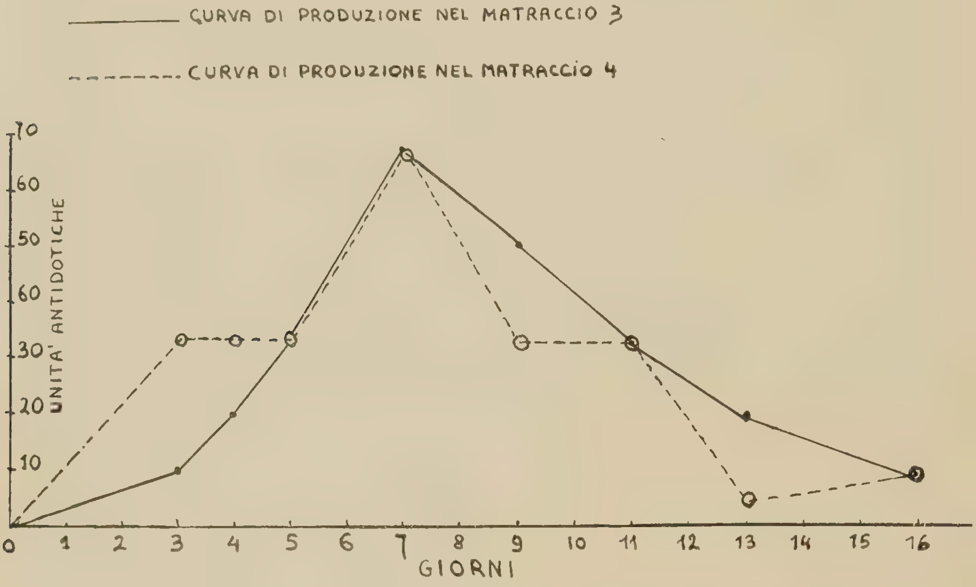
Come secondo punto si é studiata l'influenza del pH di partenza del terreno culturale sulla produzione del principio antidotico, del pH che successivamente si stabilisce, e contemporaneamente l'andamento del titolo del principio antidotico nel tempo, costruendo così la curva di produzione. Si é usato per questi esperimenti il terreno all'Abraham casidrol 1 % seminando in ogni matraccio 1,5 cc. di sospensione di spore (sospensione ottenuta unendo all'agar cultura 10 cc. di laurilsulfonato diluito 1 : 10.000). Le culture erano tenute in termostato a 28°. Il pH dei terreni era regolato con HCL e Na_2CO_3 e determinato elettrometricamente. Si sono usati i seguenti 6 terreni con i valori di pH di partenza rispettivamente di:

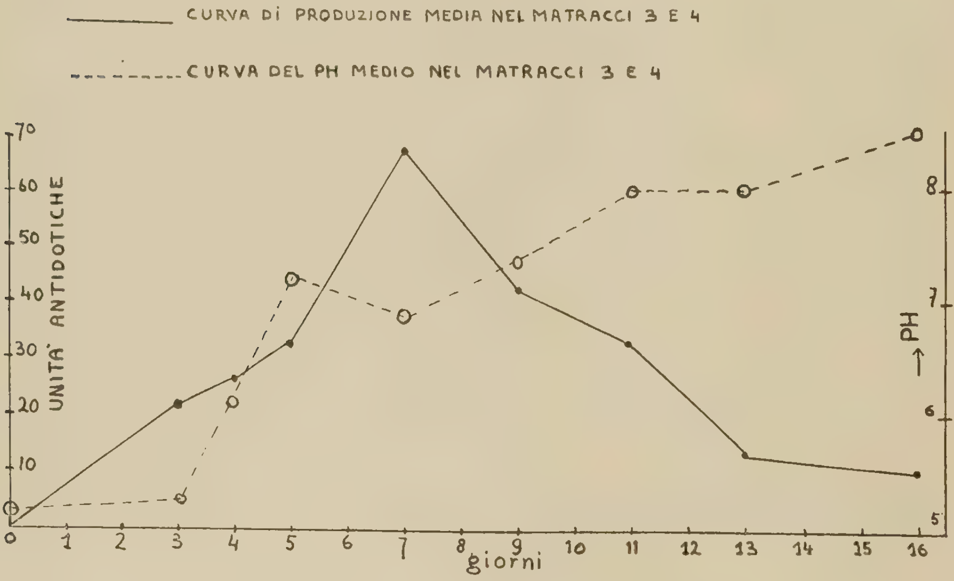
matraccio 1 pH 3,81
,, 2 pH 4,01
,, 3 pH 5,08
,, 4 pH 5,18
,, 5 pH 7,68
,, 6 pH 8

Le determinazioni vennero eseguite in 3.4.5.7.9.11.13;16 giornata per i matracci 3 e 4 e in 5 e 13 giornata per i matracci 1.2.5.6. I risultati ottenuti sono riportati nella tabella seguente:

matraccio	1		2		3		4		5		6	
	pH	U.A	pH	U.A	pH	U.A	pH	U.A	pH	U.A	pH	U.A
giorno 3	5,53		5,13		5,53	33	5,03	10	6,03		6,09	
,, 4					6,04	33	6,15	20				
,, 5	6,76	10	6,45	33	7,33	33	7,01	33	7,13	20	7,06	10
,, 7					6,05	67	7,63	67				
,, 9					7,06	33	7,73	50				
,, 11					8,03	33	8	33				
,, 13	7,98	10	8,01	5	8,03	5	8,02	20	7,63	5	7,83	5
,, 16					8,05	10	8,9	10				

Con i sopra riportati dati si sono costruite le seguenti curve:





Tutte le determinazioni del pH eseguite nei presenti esperimenti sono state eseguite elettrometricamente.

Dai risultati riportati nelle curve risulta:

1. *che la produzione del principio antidotico raggiunge un massimo in settima giornata.*
2. *che il pH di partenza influisce sulla produzione di principio antidotico, avendosi le rese migliori per pH che variano da 4 a 5.*

3. *che il pH che successivamente si stabilisce segue un andamento quasi regolarmente crescente nei progressivi giorni di cultura.*
4. *che l'optimum di resa corrisponde a un valore di pH di circa* 7.

Caratteri chimico fisici del principio antidotico prodotto dall'*Aspergillus fumigatus*

Si é proceduto allo studio di alcuni caratteri chimico fisici del principio antidotico prodotto dall'*A. fumigatus*, in modo da avere una conoscenza orientativa per un eventuale isolamento e purificazione del principio stesso.

a) Prova di resistenza al calore.

Si é scaldato il filtrato a bagno maria per 30 minuti rispettivamente a 60.80.90 gradi e inoltre a 100 gradi per 10 minuti. Nel filtrato non riscaldato si aveva un'attività pari a 33 unità antidotiche, mentre nel filtrato scaldato alle temperature suddette si ebbe in ogni caso perdita completa dell'attività. Da notare che questo risultato é in accordo con quanto osservato da Ramon e da Cavalli per il principio antidotico del filtrato di *P. notatum*, che viene pure distrutto a 60°.

b) Prova di resistenza agli acidi e agli alcali.

Per provare la resistenza agli ácidi e agli alcali si é aggiustato il pH del filtrato di *A. fumigatus* mediante acidificazione con HCL e alcalinizzazzione con Na_2CO_3: la determinazione del pH é stata fatta elettrometricamente. Il filtrato non trattato aveva un pH di 5,53 e titolva 33 unità antidotiche. Si sono stabiliti i seguenti valori di pH:

2,08 4,12 7,06 8 10

(questi ultimi valori sono stati determinati con indicatore Merk, superando il limite del potenziometro).

Le provette contenenti il filtrato regolato ai 6 diversi pH erano lasciate per 24 ore in ghiacciaia. Dopo 24 ore si fece la titolazione del filtrato e si ottennero i seguenti risultati:

pH del filtrato	titolo in unità antidotiche
2,08	5
4,12	5
5,55	10
7,06	33
8	33
10	10

Da questo esperimento risulta che il potere antidotico non viene distrutto completamente né dagli acidi, né dagli alcali; che si ha una diminuzione del titolo sia per acidificazione, che per alcalinizzazzione oltre un valore di pH 10. Che le condizioni migliori per il mantenimento dell'attività antidotica sono tra pH 7 e 8.

c) Resistenza nel tempo.

Si é provata la resistenza del filtrato di *A. fumigatus* nel tempo, mantenendo il filtrato in ghiacciaia e a temperatura ambiente. Sia il filtrato tenuto per 40 giorni in ghiacciaia, che il filtrato tenuto per 6 giorni a temperatura ambiente (23° circa) mantennero il titolo invariato.

d) Prova della dializzabilità.

Per provare la dializzabilità del filtrato di *A. fumigatus* si sono messi 10 cc. del liquido in un apposito sacchetto di cellophane, questo sacchetto é stato immerso in acqua fredda corrente per 24 ore. Il filtrato che in partenza aveva un titolo corrispondente a 50 unità antidotiche, dopo la dialisi aveva un titolo di 20 unità antidotiche. Tenendo conto della diluizione subita dal filtrato durante la prova, si conclude che il principio antidotico é una sostanza in parte solo dializzabile.

Dai precedenti esperimenti si possono trarre le seguenti conclusioni: *il principio antidotico prodotto dall'A. fumigatus é termolabile, resistente poco agli acidi e un pò di piú agli alcali, conserva il massimo di attività con un pH alcalino di 7—8, resiste nel tempo a 4 gradi e a 23 gradi, dializza solo in parte.*

Azione del filtrato di *Aspergillus fumigatus* sulla tossina difterica

E' stata studiata l'azione del principio antidotico prodotto dall'*A. fumigatus* sulla tossina difterica. In questo caso si é cercato di vedere se tale principio agisse oltre che sul potere tossico anche sul potere di combinazione della tossina difterica. Si é quindi studiato il potere di combinazione della tossina con il siero antidifterico, e precisamente si é studiato il potere flocculante della tossina non trattata e della tossina trattata con il filtrato. Per studiare le variazioni del potere tossico ho usato il metodo di Ipsen, che descriverò in seguito, e che dà il titolo di tossina in dml con precisione superiore a quella consentita da altri metodi.

Si é studiato innanzi tutto se il filtrato di *A. fumigatus* fosse tossico per la cavia; per questo si sono inoculate due cavie: nella prima si é inoculato nel sottocutaneo 1 cc. della sostanza pura; nella seconda 1 cc. della sostanza diluita 1 : 10 nel peritoneo. Entrambe le cavie sono sopravvissute. Fatti questi esperimenti preliminari, si é studiato parallelamente l'andamento del potere tossico e del potere di combinazione della tossina difterica dopo trattamento con filtrato di *A. fumigatus*. Per questo si sono poste in termostato a 37° le seguenti miscele: Miscela A: 200 cc. di tossina più 200 cc. di filtrato di *A. fumigatus*; Miscela C: 200 cc. di tossina più 200 cc. di soluzione fisiologica. Si sono eseguite due determinazioni del potere tossico e del potere flocculante, rispettivamente dopo 20 ore e dopo 3 giorni di contatto della tossina con il filtrato, a 37 gradi.

Dopo 20 ore di contatto, il potere flocculante della tossina non trattata é di 36 U.F. quello della tossina tratta con filtrato é di 28 U.F., cioé é calato del 25 %.

Le stesse miscele A e C di tossina e filtrato e tossina e soluzione fisiologica, usate per l'esperienza della flocculazione, sono state usate per la prova della tossicità della tossina difterica dopo 20 ore di contatto a 37°. Come animali da esperimento si sono usate le cavie del peso di 200—250 grammi. Per la valutazione del titolo di tossina in DML mi sono servita del metodo di Ipsen che permette di valutare anche piccole differenze di tossicità con pochi animali. A questo scopo si devono inoculare multipli di dose minima letale e osservare il tempo di morte possibilmente ogni ora. Una precedente esperienza preliminare aveva dimostrato che la tossina usata conteneva più di 300 DML per cc.

Prima esperienza

Si inoculano due animali per ogni dose usata, si determinano i tempi di morte osservando ogni ora i sopravviventi:

cc. di tossina inoculati		tempi di morte	
0,1	(1 cc. di 1 : 10)	19 ore	19 ore
0,05	(1 cc. di 1 : 20)	19 ,,	19 ,,
0,025	(1 cc. di 1 : 40)	22 ,,	24 ,,
0,0125	(1 cc. di 1 : 80)	28 ,,	28 ,,

Si scrivono quindi i logaritmi delle dosi usate:

dosi (D)	log. D
0,1	— 1
0,05	— 1,3
0,025	— 1,6
0,0125	— 1,9

e al posto di ogni tempo di morte si scrive un valore f(T) che si desume da una tabella calcolata da Ipsen e riportata nel suo libro che dà i valori di f(T) per ogni T ed ogni tossina:

$$f(T) = \log \left[\left(\frac{K}{\frac{T}{t} - 1} \right)^{\frac{1}{\alpha}} + 1 \right]$$

ove t K e α sono costanti caratteristiche della tossina in esame. Per la tossina difterica Ipsen ha calcolato in base a dati sperimentali di Glenny, che K = 2,69

$$\alpha = 0{,}5 \qquad t = 14{,}1$$

Ad esempio, per T = 20 ore, si ottiene dalla tabella di Ipsen, oppure dalla formula, f(T) = 1,2634. Si fa quindi la media dei valori di f(T) per ogni dose. Ad ogni media di f(T) si sottrae il corrispondente valore di log D tenendo conto del segno. Si ottengono così dei valori pressoché uguali per le diverse dosi, la cui media é il logaritmo del titolo della tossina in esame.

Riporto in tabella tutti i dati calcolati.

Tossicita' della tossina non trattata

Miscela C: 200 cc. di tossina più 200 cc. di soluzione fisiologica.

D	Tempi di morte		log D
0,1	19	19	— 1
0,05	19	19	— 1,3
0,025	22	24	— 1,6
0,0125	28	28	— 1,9

f(T)		$\overline{f(T)}$	$\overline{f(T)}$ — log D
1,79	1,79	1,79	2,79
1,79	1,79	1,79	3,09
1,44	1,20	1,32	2,92
0,93	0,93	0,93	2,83
			11,63 : 4 = 2,91

antilog di 2,91 = 813 DML per cc.

Tossicita' della tossina trattata con filtrato

Miscela A: 200 cc. di tossina più 200 cc. di filtrato

D	tempi di morte		log D
0,1	22	28	— 1
0,05	27	30	— 1,3
0,025	27	37	— 1,6
0,0125	36	39	— 1,9

f(T)		$\overline{f(T)}$	$\overline{f(T)}$ — log D
1,44	0,93	1,19	2,19
0,98	0,82	0,90	2,20
0,98	0,57	0,75	2,35
0,60	0,52	0,56	2,46

9,20 : 4 = 2,30

antilog di 2,30 = 200 DML per cc.

Dalle sopra riportate tabelle si vede che la tossina non trattata titola 813 DML per cc., la tossina trattata con filtrato titola 200 DML per cc., il che dimostra una netta diminuzione del potere tossico, precisamente la tossina ha perso il 75 % della sua tossicità.

In una seconda esperienza ripetuta dopo 3 giorni di contatto della miscela tossina e filtrato, il potere flocculante nella tossina non trattata era di 32 U.F.; nella tossina trattata era scomparso (la tossina non flocculava in nessuna provetta). Per quanto riguarda la tossicità la tossina non trattata titolava 400 DML per cc., la tossina trattata 74,5 DML per cc.

Gli esperimenti sopra riportati dimostrano che il *principio antidotico prodotto dall'A. fumigatus determina una diminuzione della tossicità della tossina difterica, senza per altro giungere, nelle condizioni qui usate, a una completa disintossicazione della tossina.* Nelle stesse condizioni invece, il potere flocculante é scomparso. E' difficile però pensare, dato che parte della tossicità é conservata, che il potere di legarsi agli anticorpi sia irrimediabilmente e completamente distrutto; si assiste speso infatti, in condizioni che alterano la molecola dell'antigene e dell'anticorpo, a variazioni del potere flocculante. Ciò induce comunque a pensare che l'azione del filtrato sulla molecola della tossina sia un'azione grossolana, non paragonabile, come pensava RAMON, all'azione della formalina la quale agisce soltanto legandosi ai gruppi aminici che determinano l'azione tossica nella tossina difterica e che, seconco le esperienze di PAPPENHEIMER, sono gli ε-aminogruppi della lisina.

Comunque ulteriori ricerche sulla attività di queste sostanze fornite di proprietà antidotica richiedono la purificazione del principio attivo. Occorrono per questo adeguati metodi chimici che attualmente non sono a disposizione nei nostri laboratori.

Riassunto

La proprietà antidotica di 68 ceppi diversi di miceti é stata studiata usando come test l'inattivazione della tossina stafilococcica. Viene descritto un rapido metodo di titolazione e viene definita una unità antidotica. Sono stati trovati attivi 7 ceppi di Penicilli, 6 di aspergilli, 4 di lieviti e un ceppo di *B. subtilis*. La massima attività é stata trovata in un ceppo di *Aspergillus fumigatus*. Per ottenere una maggiore produzione della sostanza attiva prodotta da questo ceppo di *Aspergillus fumigatus*, é stata studiata l'influenza di alcuni terreni culturali e del loro pH sulla curva di produzione. Migliore terreno fu trovato il terreno di Abraham con aggiunta di idrolisato di caseina al 0,5—1 %; migliore pH iniziale del terreno 4—5. Massimo di produzione si ebbe in settima giornata con pH di circa 7. La sostanza antidotica prodotta dall'*Aspergillus fumigatus* é distrutta dal riscaldamento per mezz'ora a 60°, ha una scarsa resistenza agli acidi e una forte resistenza agli alcali, con optimum di stabilità a pH 7, dializza solo in parte. Non é tossica, per la cavia, contrariamente a quanto é già noto non ha attività antibiotica verso lo stafilococco. L'azione sulla tossina difterica é stata pure studiata. Dopo 20 ore di contatto la sostanza

diminuisce la tossicità della tossina difterica da 813 DML a 200 DML e il potere flocculante da 36 UF a 28 UF. Dopo 3 giorni di contatto la tossicità era diminuita da 400 DML a 74,5 DML e il potere flocculante diretto era scomparso. Ulteriori ricerche richiedono una purificazione del principio antidotico.

Summary

The antidotal property of 68 different strains of mycetes has been studied using as a test the inactivation of the staphilococcus toxin. A rapid method of titration is described and an antidotal unit is defined. 7 strains of Penicilli, 6 strains of Aspergilli, 4 strains of yeasts, 1 of *B. subtilis* have been found to be active. The greatest activity was found for a strain of *Aspergillus fumigatus*. In order to get a larger production of the active substance produced by this strain of *Aspergillus fumigatus*, the influence of many cultural media and their pH on the production curve were investigated. The best medium was found to be Abraham's medium to which 0,5—1 % of hydrolysed casein had been added, the best initial pH of medium 4—5. Maximum production was observed on the 7th day, with pH of about 7. The antidotal substance produced by *Aspergillus fumigatus* is destroyed by heating at 60° for ½ hour, has a weak resistance against acids and a strong one against alkali, with optimum of stability at pH 7, dialyzes only partially. It is not toxic for guinea pigs; differently from what is already known, it has no antibiotic activity against staphilococci. The action on the diphteria toxin has been studied. After 20 hours of contact the substance diminishes the toxicity of the diphteria toxin from 813 DML to 200 DML, and the flocculation power from 36 U.F. to 28 U.F. After 3 days of contact the toxicity was decreased from 400 DML to 74,5 DML and the direct flocculation power had vanished. Further analysis demands, however, a purification of the antidotal principle.

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Critical Survey of the Mycological Literature of the years 1939 to 1942 [1]

by

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A. Introduction

At the request of the Editors of Mycopathologia, the first critical survey of the mycological literature of the year 1938 has been published [2]).

Preparations were made to present a similar survey for the following year, when World War II broke out in September 1939. As a consequence, the European literature was soon entirely cut off. This event has interrupted the collection of a substantial part of the pertinent literature. The war service of the reviewer in 1943—46 further delayed the completion of this work. Due to the devastation of the war not only have the scientific investigations in Central European Countries been completely stopped, but due to the destruction of the libraries great losses were suffered in the material, much of which never can be replaced.

It was the belief of the reviewer that the presentation of a survey covering the years from 1939 through 1942 would fill a gap in many parts of the world where libraries have been completely destroyed. Moreover, some of the material presented here would not have been available in some countries even in normal times.

In the year 1948 the American Association for the Advancement of Science celebrates its first centenary on the keynote of ,,One World of Science''. The present review has the same underlying idea: to show the development of ,,One World'' in medical mycology, to promote cross fertilization of ideas, to better acquaint research workers in far off countries with the result of each others' investigations, to reveal the newer tools so necessary for further investigations.

However, this high goal could not be achieved. The reader will notice the almost complete lack of references to the Slavic literature. This was absolutely not available either in the original or in abstracts. In this respect, the often quoted ,,iron curtain'' has cut the world into two parts. Before the World Wars, Russian and other Slavic literature had been published as original contributions in German and French scientific journals. One could get an impression and even a good idea of scientific research, and one could get a fairly good picture of the main ideas of the investigations revealed in these publications. Since 1933 this international mutual exchange of scientific ideas has been interrupted. There is a Slavic and a Non-Slavic world.

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[1]) By request of the Editors.

[2]) BENEDEK, T.: Critical survey of the mycological literature of the year 1938. Mycopathologica II (4): 267—316, 1940.

This is, however, only one aspect of the lack of ,,One World''.

Reviewing a substantial number of pertinent scientific papers (over 500) one got the definite impression that the knowledge of and the interest in foreign language (foreign to the writers) scientific papers is almost non-exsistent. That is a fair inference in view of the bibliographies quoted. The language barrier to the exchange of ideas, seems to have gained and be gaining more and more momentum, causing insurmountable obstacles. Central European publications hardly know anything about the valuable and highly flourishing South American (Spanish, Portuguese) scientific production, although Argentinean and Brazilean writers seem to have first rate knowledge, at least, of the English language publications. Reviewing the world literature in our special field one gets more and more the impression that the international one world of science is cut up into parochial states in which the area of one national language is also the delimitation of the inbred research which does not seek, nor even intend, to enter into an exchange of ideas beyond their own borders.

Within the parochial states, within the limits of national languages there is another serious obstacle in the way of progress. This is a deep-rooted fear and lack of scientific courage to touch upon ,,controversial'' topics, to discuss the results of the research of others, to engage in objective discussion of the concepts of the contrary-minded. They believe that haughty silence will keep the lid tight on newer, progressive concepts. They believe that continuous repetition of the same fallacies will turn them into truth.

In the hope of bringing into one focus the medical mycological production of widely separated geographical areas, and beyond that, to present the achievement of one language group to another one, which without the use of a lingua franca — here the English — would not be possible, this review was compiled.

There are, however, a few important points which painfully come to the fore while reviewing the medical mycological contributions through several years in many languages.

These are, at least, three in number: (1) the progressive loss of an accepted uniform nomenclature in medical mycology; (2) the lack of any scientific yardstick similar to KOCH's postulates for the evaluation of the etiologic role of mycological findings, which represent at least a good working concept; (3) the uncritical adherence to false concepts which are thoughtlessly repeated for decades without any scientific urge to check and recheck them.

This critical attitude has not only a purely academic value, but also has a profound practical significance if and when the results of mycological research should be applied in the healing art. As in the physical sciences one has to have the urge to verify time and again older concepts which even as a working hypothesis proved themselves to be false.

We have to turn now to the discussion of the three basic problems recognized as important for the sound future development of medical mycology.

(1) The reviewer has dealt at length with the first of these problems [1]).

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[1]) BENEDEK, T.: On the newer trend of classification of Dermatophytes. Mycopathologia IV (3): 201—206, 1948.

It was pointed out that "the international understanding in medical mycology leaves much to be desired. From nation to nation, from the area of one language to the area of another, the classification of the dermatophytes changes to such an extent that only the most initiated could follow up the developments in their classification that have taken place during the last decades, or can wade through the Babel of fantastic mycological terms."

(2) The lack of critical evaluation of mycological findings.

David Gruby's fundamental discovery of all but one of the major human pathogenic fungi in 1841—44 met with complete incredulity from part of the leading contemporary dermatologists and his findings were stamped as "fantasies of the microscopist." This propaganda proved so successful that Gruby's discovery fell into oblivion for half a century. Sabouraud — fifty years later — rediscovered the same fungi and, still against stubborn resistance, finally proved to a better prepared and more receptive generation of dermatologists that microscopical plants can cause human disease. At about the centenary of Gruby's discovery [1]) medical mycology reached its height. But unmistakable signs of decay which may discredit everything which former generations created, have put in their appearance.

It will be the arduous task of the third half century of medical mycology to clean house, to eradicate enormous fallacies and to reestablish sound evaluation of mycological findings.

(3) The uncritical adherence to false concepts concerning the etiologic and pathogenetic role of fungi comes most conspicuously to the fore in three groups of conditions stamped as "fungus infections" (*a*) dermatophytosis, (*b*) onychomycosis and (*c*) otomycosis.

(*a*) One grotesque example of this decay of any etiological thinking and of any pathogenetic concept can be found in the fungus theory of the so-called "dermatophytosis." Montgomery and Casper [2]) presented not so long ago typical examples of this decay of etiologic research and pathogenetic concepts. They *assigned* different species of fungi as etiologic agents to the different clinical forms of the cutaneous manifestations of a hematogen-endogenous eruption, i.e. pompholyx, without the least embarrassment by the complete ignorance of the classical evidences presented in this matter. In "dermatophytosis" of the palms and soles certain clinical types (eczematous and hyperkeratotic forms of pompholyx) are caused by *Tr. gypseum*, another type by *Tr. purpureum*, a third type by *E. inguinale*. They carry ver the very same principle to involvement of the nail organ, typical examples of pompholyx of this structure which are stamped by them as "onychomycosis." One clinical form is caused by *Monilias*, another by *Tr. gypseum* or *Tr. purpureum*.

The superficiality and absurdity of this type of "etiological" investigation can be illustrated best by comparison with syphilis if someone were to assume that every clinical type of syphilis, be it, for instance, "lichenoid," "rupioid", "ulcerative-gummatous," is caused by different species of microorganisms.

(*b*) *Onychomycosis* — if we understand, in sensu strictu, the primary, fungus infection of the nail organ — is a rare disease. Sabouraud [3]) underscored this fact in his Les Teignes, emphasizing that he found only one single case of onychomycosis among 500 consecutive cases of fungus infections.

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[1]) Cf. Zakon, S. J. and Benedek, T.: David Gruby and the centenary of medical mycology, 1841—1941. Bull. Inst. Hist. Med. (Baltimore), 1942.

[2]) Montgomery, R. M. and Casper, E. A.: Cutaneous manifestations of the fungi causing dermatophytosis and onychomycosis. J.A.M.A. 128 (2): 77—83, 1945.

[3]) "A Paris, l'onychose trichophytique est rare. J'ai pu examiner 500 cas de dermatomycoses sans rencontrer plus d'un sujet qui en fut atteint." "Le plus souvent il s'agit de cas sporadiques chez l'homme adulte et leur cause directe reste inconnue." "Tous les auteurs sont d'avis que, l'onychose trichophytique est ordinairement secondaire a une lésion préalable de l'épiderme de la main." Sabouraud, Les Teignes, pp. 452—453, Paris, Masson et Cie, 1910.

Among 317 consecutive cases of more or less severe instances of dermatomycoses observed by this reviewer on Okinawa [1]) in a sub-tropical climate, not a single case of onychomycosis was noted.

What is commonly designated by dermatological texts as "onychomycosis" is etiologically and pathogenetically not a fungus infection of the nail organ, but the manifestation of a hematogen-endogenous process, i.e. pompholyx, caused by the *B. endoparasiticus Benedek*, 1927.

In the scrapings and in the detritus material of these involved nails a great variety of nosoparasites may be present and, thus, are easily demonstrable in culture. When hyphomycetes are cultured (even common molds, like *Penicillium, sp., Aspergillus, sp.*, etc.), the condition is usually called "onychomycosis"; when yeastlike organisms are found, it is "moniliasis"; if staphylococci are recovered, it is "staphylococcic onychia or paronychia."

(*c*) "*Otomycosis*" is carried in the literature since 1883. This is probably the most glaring illustration of the inertia of half-truth, and half-knowledge with which a label perpetuates itself in the medical and mycological literature.

Siebenmann (1883) has been responsible for introducing the concept of "otomycosis," supposedly caused by *Penicillium, Aspergillus* and a host of other common and harmless molds. It was understandable that during the infancy of medical mycology the simple presence or growth of any fungi from the detritus of the pathologic process was not only suspected but directly made the "causative" organism of the dermatological condition of the external ear and external auditory canal.

Since Siebenmann's publication (Ztschr. f. Ohrenh.. 12: 124—161, 1883) innumerable communications repeated and presented similar findings without any critical evaluation of the material.

In these cases of so-called "otomycosis" there has never been offered the slightest scientific evidence which might be considered conclusive. The simple presence of the fungi, *Penicillium, Aspergillus, Mucor*, etc. in the desquamating epithelial debris of the external auditory canal, let alone the presence of fungi in the acid ceruminal plug, does not make these harmless molds pathogens or the direct cause of the existing dermatitis.

What is commonly called by otologists "otomycosis" of the external ear and the meatus externus (which is covered by the continuation of the same epidermis which covers the cartillage of the ear) is again not a fungus disease, but is *seborrheic dermatitis*.

The papers critically reviewed here reveal a sad lack of formal training in medical mycology among otologists, which is superimposed on the ignorance of the commonest skin conditions of the external ear. For example, "*athlete's foot of the nose*" (cf. Felderman, 1940) was even offered as a serious, scientific, diagnosis.

The reviewer had the opportunity to examine clinically and mycologically a very great number of cases of otitis externa involving the pinna and the external auditory canal. One could culture from the desquamating epithelial debris and from the inspissated, dried, exuding serum as well as from the acid ceruminal plug almost everything, bacteria, yeastlike organisms and common, harmless molds like *Penicillium, Aspergillus, Mucor*, etc.

The ease and simplicity with which ubiquitous fungi recovered from debris of the pathologic process are stamped pathogens and made *the* causative agents of an entirely different process is amazing.

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[1]) Benedek, T.: Pompholyx, its etiology, pathogenesis, and vaccine therapy, with particular reference to its military importance and post-war significance. Urol. & Cutan. Rev. 50 (8): 467—493, 1946.

These grave and entirely unnecessary diagnostic errors have not only a purely academic import. They show the most deleterious consequences in the therapy of these conditions, i.e. "dermatophytosis," "onychomycosis," "otomycosis."

It is high time to become clearly aware of these facts. Errors of the past based on fallacies of eminent authorities of their time are carried on without the slightest scientific control or criticism. Many times these opinions have been and are found to be the direct opposite of the real truth.

B. Hyphomycosis

(*a*) *Technic*

AREA LEAO (1939) contributed to the *Mycological Technique* in evolving a new method of examining hair, scales, etc. After a critical review of the classical methods of mycological examination of hair, scales, etc., mainly SABOURAUD's procedure, he points out their inconveniences, the waste of material caused by potassium hydroxide solution which destroys the interrelationship of the parasite and of the material parasitized. He recommended the following technique:

1. Place the material to be examined (hair, scales etc.) on a clean slide or, better, into a small watch glass, and over it with the following mixture freshly prepared:

Hydrogen peroxide, 12 vols.	9 parts
Ammonia	1 part

2. Heat mildly on Bunsen burner until steaming, or leave for several hours exposed to laboratory temperature. Decoloration of the material takes place which may be seen under the microscope.

3. At the end of the decoloraion of the material which occurs more quickly at high temperature, pour off the mixture of hydrogen peroxide and ammonia in order to dehydrate the material, treat it repeatedly with absolute alcohol on the slide itself, then let it pass through xylol and mount in Canada balsam. The preparation is thus better preserved. Yet, it may also be mounted in lactophenol, the edges of the coverglass having to be varnished. For staining the material, after treating it with the mixture of hydrogen peroxide and ammonia, it is enough to wash in distilled water and treat by the usual methods of staining, and then dehydrate and mount in balsam.

The method furnishes preparations of great neatness, producing a great differentiation of the parasite and preserving the material without alteration, mainly hairs which remain uninjured and show their entire structure. The method is also fit for examination of cultures, mainly pigmented ones. When there is formation of black sclerotia, thanks to the thorough decoloration produced by this procedure, the fructification apparatus and mycelian woof are better discernible.

FISHBACK and GREGERSEN (1939) recommended *a permanent method of labelling microscopic slides.*

After mounting tissues or other material the blank end of the slide is wiped off with ether. Data can then be written on the glass with India ink, or stamped on it. After the ink dries the data space is brushed over quickly and lightly with clear lacquer applied with a camel's hair brush. For a contrast background the reverse of the above space is covered with white lacquer.

It dries in a few minutes. A special lacquer thinner is used to keep the lacquer at proper consistency, and to wipe off any excess.

Lanternslides may be labelled similarly, or arrows inserted for regional emphasis, with India ink covered by the clear lacquer. Reagent bottles, or other glass vessels, require a primary background coat of white lacquer put on the label area, and upon this the legend is stamped, then covered by clear lacquer. India ink written upon the white lacquer tends to crack as it dries.

SZODORAY (1939) called attention to a *staining method of fungi* in scales with special regard to BERBERIAN's method. He tried this staining method in 40 cases. The method of BERBERIAN consists in staining of defatted scales with Toluidin MARTINOTTI and subsequent differentiation in 0.5 % glacial acetic acid. The method is easier to be carried out than GRAM's method and the spores and mycelia are well stained. Especially good results were obtained in *Epidermophyton-mycelia, Microsporon furfur,* favus. In scales of pityriasis versicolor taken eight years before, by means of this staining, details of the fungal elements showed up satisfactorily.

BENEDEK dealt with the use and application of photography and photomicrography in mycology.

He described (1939*a*) two useful devices for the photography of mycological or bacteriological specimens. The one is a plaster of Paris mould to take up giant cultures, the second is a holder for test tube cultures. The latter is provided with a spirit level on the top and with a nut below to permit using a tripod. A description is given of the use of the plaster of Paris mould in taking Kodachrome pictures with the Leica camera in connection with the accessory setting device "Beooy" of Leitz.

In a second paper (1939*b*) he gave a short description of the mycologic stage clamps which have made test tube cultures of microscopic fungi available for purposes of photomicrography. A special method of inoculation of these test tube cultures is recommended. The most appropiate photomicrographic equipment for mycological purpose is discussed. The large photographic camaras are considerd to be inappropriate and unnecessary. The author favors the use of miniature camaras (Leica, Contax) and calls attention to the useful novelties in this field (Zeiss Planachromats, Zeiss Homals) which make it possible to obtain a perfectly plain field and a correct definition. Finally an open request is made to American Optical manufacturers to make the necessary change on the rear mount of the eyepieces of the excellent American microscopes which would allow them to be used with the miniature camaras Leica and Contax.

ADAMS (1940) suggested a *Procedure for Staining Filamentous Algae and Fungi on the Slide.*

The Venetian Turpentine method for mounting delicate structures such as filamentous algae requires so much handling of the bulk material that tangling, twisting, and excessive breaking of the filaments is almost unavoidable. The conjugating filaments of forms which show scalariform conjugation are likely to be separated and otherwise distorted. Due to matting of the filaments, uniform destaining is difficult of accomplishment, and satisfactory teasing-out of the matted filaments in the thick Venetian Turpentine just prior to covering is difficult or impossible.

The following procedure, based upon the use of a modification of Haupt's gelatine fixative, has been used successfully in overcoming these difficulties and in shortening the preparation time.

Dissolve 1.5 g of a good grade of gelatin in 100 ml. of distilled water at 30—35° C. Add 5 ml. of glycerin and 2 g. of phenol. Smear a thin film of the fixative upon a warm slide.

After killing and washing the bulk material in running water remove enough for a slide with fine forceps. Place this upon the smeared slide with a "dragging" movement, beginning at one end of the slide and pulling the filaments toward the other end. The water which adheres to the filaments is sufficient for floating them into position; a large quantity of water such as is used for floating paraffin ribbons is unnecessary and undesirable. Most of the filaments will thus arrange themselves in a free and parallel fashion. Conjugating filaments can be mounted in this manner without damage. If desired, long ends extending beyond the area to be covered may be turned in beside the main mass of filaments. Invert the slide over a shallow dish containing a small quantity of commercial (40 %) formaldehyde and cover with an inverted dish or low bell jar. Glass staining dishes of the low, rectangular form, slightly less than 3 inches wide, serve this purpose well. Leave the slides thus exposed to the fumes of formaldehyde for ½ hour. Wash slides in distilled water, 5—10 minutes. They are now ready for staining in aqueous stain or for the alcohol series if an alcoholic stain is to be used. Clear in xylene and mount in balsam.

The following schedule has given good preparations of Spirogyra:

1. Kill and fix in chromo-acetic acid (1 % chromic acid, 3 % glacial acetic acid), 12 hours.
2. Wash in running water, 12 hours.
3. Fix material to slide as described above. Wash in distilled water, 5—10 minutes. (Slides may at this point be run up to 85 % alcohol for hardening and returned to water.)
4. Mordant in 2 % aqueous solution of ferric ammonium sulfate, 4 hours. Wash in several changes of water, ½ hour.
5. Stain in 0.5 % aqueous hematoxylin, 12 hours. Wash in water (2 or 3 changes), ½ hour.
6. Destain with 1 % aqueous solution of ferric ammonium sulfate, about 1 hour. Wash in water, ½ hour.
7. Dehydrate by closely graded series of alcohols to 95 %.
8. Counterstain with 0.5 % orange G in 95 % alcohol, 15—30 seconds.
9. Complete dehydration in absolute alcohol, 1—2 minutes. Clear in xylene. Mount in balsam.

De Almeida and Da Silva Lacaz (1940) reported on a *New Technic for the rapid demonstration of Ascospores.* Their medium consists of 5 grs starch in 1.000 cc of distilled water. Sterilize at 120° C and pour out in test tubes. The advantage of this medium is its easy preparation and its great usefulness on account of causing quick ascospore formation. If the strain studied should develop ascospores, it will do so already at the end of 24 hours in abundance at 37° C in the incubator.

Maneval (1941) reported on *staining bacteria and yeasts with acid dyes.*

Various acid dyes proved satisfactory for the routine staining of bacteria. Those used are acid fuchsin, anilin blue w.s., fast acid blue R, fast green FCF, light green, orseilline BB, erythrosin, phloxine and rose bengal. Acid fuchsin, fast green, anilin blue, and orseilline are especially recommended. Phenolic solutions of the dyes, acidified with acetic acid, with the addition of ferric chloride to those containing acid fuchsin, anilin blue, fast green or light green, are used. Procedures are given in detail for staining or demonstrating vegetative cells, resting and germinating spores, capsules, sheaths and glycogen in bacteria; germinating and conjugating spores of yeast; and for counterstaining after acid fast or Gram staining. The principal advantages of using acid dyes are better differentiation, and less tendency for slime and debris to take the dye.

The organisms stained included mainly *Alcaligenes viscosus, Bacillus cereus, Bacillus megatherium, Bacillus mycoides, Bacillus subtilis, Bacterium coli,* clostridia in mixed cultures, *Pseudomonas pyocyaneus, Rhizobium melitoti, Rhizobium trifolii, Rhodococcus roseus, Saccharomyces ellipsoideus, Sarcina lutea, Serratia marcescens, Spirillum rubrum, spirochaetes,* etc. from the mouth, *Staphylococcus citreus,* and *Streptococcus lactis acidi.*

Formulae

Solution A.	Phenol, 5 % aqueous.	30 cc.
	Glacial acetic acid, 20 % aqueous	8—10 cc.
	Ferric chloride, 30 % aqueous.	4 cc.

Add a 1 % aqueous solution of any one of the following dyes in the quantities indicated: acid fuchsin (1—2 cc.); anilin blue w.s. (2—8 cc.); fast green FCF (2—8 cc.); light green (2—8 cc.); fast acid blue R (15 cc.) omitting ferric chloride; orseilline BB (8 cc.) omitting ferric chloride.

Solution B.	Phenol, 5 % aqueous.	100 cc.

To solution B add 1 gram of rose bengal, erythrosin or phloxine, previously dissolved in about 5 cc. of distilled water. Then add drop by drop enough (6—8 drops) 20 % aqueous acetic acid to cause a trace of cloudiness. Finally add 20 to 50 cc. of 95 % alcohol. This seems to improve the solution and if a slight cloudiness has developed it should disappear; if not add a trace of ammonium hydroxide in 95 % alcohol.

Mixed solutions of dyes. Acid fuchsin (solution A plus dye) may be added to either fast green (A plus dye) or light green (A plus dye) in the proportion of 1 part acid fuchsin to 4 parts of the other; or enough acid fuchsin may be added to the green dye to produce a deep reddish purple color. This mixture stains a greenish blue to nearly purple. It has considerable selective action too, especially if applied for only a few seconds. Spores, for example, may stain light pink and the mother cells green to purple.

In general aqueous or phenolic solutions of acid fuchsin, anilin blue, fast green, or light green stain bacteria very lightly or scarcely at all. The use of the ferric chloride is therefore very important but the part it plays is unknown.

Staining procedures. Vegetative cells. In general the procedure for staining vegetative cells of bacteria with acid dyes is to prepare a smear in a drop of water, dry without heating, stain 10 seconds to 1 minute, wash with water and dry. A longer time (2—3 minutes) is better with the fluorescein dyes and with fast acid blue R. Staining is very good and clear-cut with fast green, acid fuchsin, anilin blue w.s., fast green and acid fuchsin mixed, or with acid fuchsin followed by fast green (about 5 seconds). The last procedure gives results similar to those with acid fuchsin and fast green mixed, but by varying the time properly one may obtain more differentiation. This differentiation may frequently be improved by treating 1 minute with Gram's iodine and washing.

Spores. Spores of bacteria may be demonstrated very well with acid dyes. Young spores will stain deeply but mature or old ones only lightly. The cells containing spores, however, can always be stained by the following technic to give a sharp contrast: Treat smears 3 minutes with solution A; wash with water, stain 3—5 minutes with acid fuchsin or fast green, singly or mixed, orseilline or rose bengal; wash with water and counterstain with fast green 2—4 seconds after orseilline, 1—2 seconds after rose bengal, and

5—8 seconds after acid fuchsin; or after fast green counterstain 10—30 seconds with rose bengal (50 % alcoholic) or orseilline.

Capsules and Sheaths. Another use of acid dyes is for demonstrating capsules or sheaths of bacteria. Films may be made in the usual way; or if there is little slime present it is best first to spread a very thin layer of albumen fixative (MAYER's) on the slide, then to add a drop of water and to make a smear. If albumen fixative is used, one should treat the preparation after drying with absolute alcohol and dry to coagulate the albumen. In either case staining should be with acid fuchsin, or with acid fuchsin and fast green mixed; or the film may be treated with solution A, washed and stained with orseilline or rose bengal.

Glycogen. If 3 or 4 beans are covered with about 2 inches of water in a preparation dish and allowed to incubate for 2—6 days, many clostridia will be found inside and around the decaying seeds. This is very good material for demonstrating the occurrence of glycogen, as well as stages in spore formation, in bacteria. A smear may be made on a slide, dried, stained very rapidly with orseilline, rose bengal, or fast green, washed, treated with GRAM's iodine for 1 minute, washed and dried. The dye stains the part of the cell that is free from glycogen, while the glycogen appears a reddish brown. Depending on age the whole cell, or all but a small part of it at one end, may give reaction for glycogen. If a young spore is present in the cell only that part between the spore and one end of the cell will give the glycogen test.

Yeasts. The mature spores of yeasts are easier to stain than those of bacteria, and stain readily with acid dyes. Spores of Saccharomyces ellipsoideus develop rather abundantly in cultures grown for a week or more at room temperature and then kept in a refrigerator for several months. Or they will develop in two weeks or less on agar prepared according to the following formula (MANEVAL, 1924): water, 1000 cc.; Liebig's beef extract, 3 g.; sodium chloride, 5 g.; dextrose, 2.5 g.; agar, 15—20 g. The spores may be stained with acid fuchsin or fast green; or with orseilline or rose bengal after treatment for 3 minutes in solution A. Then they may be washed with water, dried, and a very thin layer of 1 % aqueous nigrosin applied. Such preparations show the vegetative cells and spores stained and the asci colorless. If, however, a counterstain is used before applying nigrosin, fast green (2—8 seconds) for the red dyes or rose bengal (20—30 seconds) for the green, the vegetative celles will be stained with the counterstain.

Saccharomyces ellipsoideus is a favorable organism for demonstrating germination and conjugation of spores. If transfers are made from old cultures containing an abundance of spores to potato dextrose agar slopes, and incubated at room temperature for 5—7 hours, many spores will germinate. Or spot inoculations on hardened drops of agar on slides as described above for bacteria, may be made and, after incubation in a moist chamber, treated as follows: stain with acid fuchsin and fast green mixed, or with acid fuchsin followed by fast green, wash, dry, apply a thin layer of aqueous nigrosin and dry. The ascospores, as described by MARCHAND (1913), may swell and develop vegetative cells directly; or they may enlarge and develop beaks which unite forming conjugation tubes, so that when two ascospores have conjugated they appear somewhat like a capital U or heart-shaped. When these resulting zygospores germinate, buds may form anywhere on them but most frequently on the conjugation tube.

Acid dyes have been used for some time in lactophenol mounts of various kinds of organisms, especially fungi. It is evident from the staining procedures and results described here that a number of acid dyes may be used also as general bacteriological stains, and that for certain purposes they are to be preferred to basic dyes. Actually it is possible to perform quite satisfactorily most or all of the staining described here with only two acid dyes, acid fuchsin and fast green.

SIMÕES BARBOSA (1941) developed a *technic to culture fungi on slides.* Following the well known technic of DE BEURMANN and GOUGEROT, NEGRONI, REDAELLI he suggested for the study of imperfect fungi with fragile conidial structure and rapid and easy growth the following simple method to obtain demonstrative preparations. The technic is as follows:

The bottom of a PETRI dish is covered with filter paper. Two slides are laid upon it for the support of three coverslips. Make filter paper humide with distilled water after the slides with PETRI dishes are autoclaved. The coverslip will stick to the slides. Start out from a culture, few days old, and grown on a liquid medium. After this culture medium is thoroughly shaken, transfer a small portion of the culture to the first coverslip, from this first one transfer some material to the second coverslip and make a third transfer from the second coverslip to a third one. Subsequently the coverslips can be removed for mycological or histological studies on different days in accordance with the growth of the culture, with the number of days or with the quantity of the inoculated material.

YEGIAN and KURUNG (1941) made interesting observations on *dextrose yeast extract medium for the isolation of fungi from sputum.*

SABOURAUD's medium with a pH of 5.5 is in general use for the isolation of fungi from the sputum; however, it inhibits only to a limited degree the growth of bacteria commonly found in the sputum when incubated at a temperature of 37° C. One or more transplants may be required before the fungus is finally recovered in pure culture. The growth of the bacteria may be further inhibited by gently swabbing the surface of the medium with a tenth-normal solution of hydrochloric acid previous to the implantation of the sputum. The application of the acid to the medium does not interfere with the growth of fungi. The foregoing observation suggested the advisability of materially increasing the acidity of SABOURAUD's medium for the recovery of fungi from the sputum.

The following simple liquid medium is suggested:

Dextrose	8.0 Gm.
Sodium chloride	1.7 Gm.
Yeast extract (Difco)	0.5 Gm.
Distilled water to make	200 c.c.
Adjust to pH 4.0	

The above medium should not be diluted when determining the pH, since it is buffered to only a slight extent if at all, and the addition of water would decrease the hydrogen-ion content.

The experiments here outlined indicate the relative value of Sabouraud's medium with pH values of 5.5 and 4.0, and dextrose-yeast-extract medium with a pH of 4.0 in the recovery of fugi from the sputum.

Fifty specimens of tuberculous sputa were collected in sterile PETRI dishes. A purulent portion from each specimen of sputum was implanted on these three media. The implanted medium was incubated at 37° C. and observed daily for a period of ten days. Sixteen of the fifty specimens of sputa yielded cultures of *Monilia* on each of the three media. In no instance on SABOURAUD's medium with a pH of 5.5 were the colonies of *Monilia* found in pure culture; but numerous colonies of bacteria were present on all fifty plates. The number of bacterial colonies was greatly reduced on SABOURAUD's medium with a pH of 4.0, and nine of the sixteen specimens gave pure cultures of *Monilia*. Sputum implanted in fifty tubes of dextrose-yeast-extract medium gave no bacterial growth as determined by cultures on nutrient agar plates, and the *Monilia* was recovered in pure culture from sixteen tubes.

Cultures of various fungi (*Coccidioides immitis, Aspergillus fumigatus, Aspergillus niger, Oidium, Monilia albicans, Geotrichum, Cryptococcus, Torula, Streptothrix, Actinomyces*, and *Sporotrichum*) were intimately mixed with sputa from tuberculous patients and implanted on SABOURAND's medium with pH values of 5.5 and 4.0, and on dextrose-yeast-extract medium with a pH of 4.0. Colonies of each of the above-mentioned fungi were found on all three media. However, many bacterial colonies were present on all the plates on SABOURAUD's medium with a pH of 5.5. The bacterial colonies were decidedly fewer in number on the SABOURAUD's plates with a pH of 4.0, while on the dextrose-yeast-extract medium with a pH of 4.0, no bacterial growth was demonstrated. The above experiments indicate that the liquid dextrose-yeast-extract medium with a pH of 4.0 is superior to the solid Sabouraud's medium for the isolation of fungi from the sputum.

The dextrose-yeast-extract medium here described is composed of a few simple ingredients, is easily prepared, and affords a ready means for the recovery of fungi from the sputum.

GRAY (1941) suggested a *solution for staining differentially the spores and vegetative cells of micro-organisms.*

A solution for staining differentially the spores and vegetative cytoplasm of bacteria, yeasts, and certain fungi has been developed. The solution is a mixture of two phenyl methane dyes, malachite green and basic fuchsin; it can be used as a concentrated aqueous solution or as a dilute saline solution. It is not necessary to use heat, though heating is recommended for staining "ripe" spores of bacteria. Decolorization and counterstaining are not required. Bacterial and yeast spores are stained blue or greenish-blue, vegetative cytoplasm light violet or pink. Young bacterial cells stain a deep violet, older cells light violet; the solution can thus be used as a general stain. The acid-fast organism *Mycobacterium berolinense* was stained greenish-blue with the granules violet. The saline solution is recommended as a differential stain with young colonies of *Aspergillus* and *Penicillium*; the terminal growing tips and young branches of hyphae are stained blue, older hyphae light violet, spores and conidiophores blue.

Solution A. The Aqueous, Concentrated Solution

The solution is composed of the following amounts of the dyes in distilled water:

Malachite green	0.50 %
Fuchsin, basic.	0.05 %

It has been found convenient to prepare separate solutions of the two dyes, in double strength; solution may be hastened by placing the containers in a water bath at 56° C; equal volumes of these solutions are mixed to obtain the staining solution with the dyes in the proportions stated. The staining solution appears to be stable. It should be noted that as there may be differences in dye content in dyes of different manufacture, or in different batches from a factory, a slight modification in quantities may be necessary. The malachite green used in this laboratory was supplied by The British Drug Houses, Limited, Toronto; the basic fuchsin by Coleman and Bell.

Solution B. The Saline, Diluted Solution

This is prepared by diluting two parts of solution A with eight parts of a 0.8 % sodium chloride solution. This mode of dilution was adopted since it was found that dilution of the aqueous, concentrated solution with water, or 10 % glycerine solution, rapidly altered the cellular contents of the fungi used.

Two yeasts, *Saccharomyces cerevisiae* and a *Saccharomyces* obtained from fermenting apple juice, were found to form spores rapidly if grown on slopes of Bacto tryptone glucose-extract agar at 35° C.; spores began to develop in 48hr.; and after three weeks about 80 % of the cells of the latter organism had formed ascospores.

In staining, the spores and vegetative cells are adequately differentiated by mixing a loopful of solution A with the yeast and placing a cover glass on the suspension. Many cells are stained a deep violet; the spores are greenish-blue; but they may not all stain simultaneously. Some cells do not stain. It has been observed that by this method, only one of the two or three spores in a large number of asci were stained.

The spores are well differentiated from the other cells by mixing the cells in the staining solution A, and allowing the film to dry, as described above. The fixed film, heated, gives better differentiation, with either solution.

After staining with solution A, it is necessary to use nigrosin in order to delineate the cell walls.

Aspergillus and *Penicillium*

The saline, diluted solution (B), was developed especially for use with "colonies" of these organisms grown on the surface of agar in plates. A clear medium is required; on Bacto potato dextrose agar, colonies were in suitable stages of development after incubation for 40 hr. at 35° C. *Aspergullis* is the better organism for demonstration since *Penicillium* forms a rather compact growth.

In staining, a drop of the solution is placed on a cover glass; this is inverted over the colony and pressure gently applied. The conidiophores and young spores stain greenishblue, the terminal growing points of hyphae the same color; older portions of hyphae become light violet, with young branches the same color as the terminal portions. Ripe spores of *Aspergillus oryzae* remain brown with a tinge of green in the contents.

(*b*) *Statistics*

MU and KUROTCHKIN (1939) presented a detailed *statistical and mycological study of dermatomycoses* observed in Peiping. In a period of 13 years, from June 1925 to June 1938, the total number of dermatological patients seen in the skin clinic of the outpatient department of the Peiping Union Medical College Hospital was 36,847. The clinically diagnosed cases of dermatomycoses constituted 16.3 per cent of the patients with cutaneous diseases, and the microscopically proved cases comprised 6.9 per cent of them. The data used here were secured from a statictical analysis of 92 cases of favus of the scalp, 1,104 cases of tinea of the scalp, 760 cases of tinea of the body, 526 cases of tinea of the hands and feet, and 49 cases of tinea of the nails.

Comparing the clinical impression vs. microscopic diagnosis of dermatomycoses, there were 6.013 individuals who showed clinical signs of fungus diseases of the hair or skin. In 2,531 of them spores or mycelia were found in the infected tissues under the microscope. Clinical evidence of favus of the scalp was found in 110 persons (1.83 %), and in 92 of them the fungus was demonstrated by microscopic examination of the affected hair (3.67 %). The clinical impression of tinea of the scalp was recorded in 1,493 cases (24.83 %), and in 1,104 of these, the diagnosis was confirmed microscopically (43.61 %). The number of individuals showing clinical signs of tinea of the body was 1,273 (21.17 %) and in 760 of them fungi were found under the microscope. There were 3,024 patients diagnosed clinically as cases of tinea of the hands and feet (50.29 %). In 1,245 cases the infected tissues were examined microscopically, and in 526 of them, fungi were demonstrated (20.77 %). A diagnosis of tinea of the nails was made in 113 cases (1.88 %). In 82 cases microscopic examination was made, and in 49 of them the clinical diagnosis was supported by microscopic findings (1.93 %).

In the 2,531 cases studied, there were 2,501 Chinese and only 30 foreingers.

Concerning the *sex*, favus of the scalp was 17.4 times more frequent in males than in females. In tinea of the scalp, the sex ratio was 25.3 : 1 in favor of the male. In tinea of the body, the incidence was 5.7 times higher in the male than in the female. The number of the male patients affected with tinea of the hands and feet was 2.9 times greater than that of female patients. Tinea of the nails occurred 2.3 times more frequently in males than in females.

The data necessary to correct the sex distribution on the basis of the standard distribution of sex among the dermatological patients who came under observation from 1925 to 1938, were not available. It is, however, shown that the ratio of male to female dermatological patients seen in the academic year 1937—1938, was 2.12 : 1. If the sex ratio of the year 1937—1938 is used which even though does not represent the actual sex incidence in the 13 year period, nevertheless it is approximately correct — the corrected ratio of male to female is 8.2 : 1 in favus of the scalp, 11.9 : 1 in tinea of the scalp, 2.7 : 1 in tinea of the body, 1.4 : 1 in tinea of the hands and feet, and 1.1 : 1 in tinea of the nails.

Favus of the scalp attacked chiefly children and young adults. Among the males, 57 per cent of the cases were patients under 15 years of age. Among the females, the disease occured 4 times more frequently before the age of 15 than after that age. In both sexes, this disease was not observed before the age of 5 or after the age of 30.

Tinea of the scalp was a disease of youth and was usually met with between 1 and 15 years of age. Among both the males and females, the 6—10 year class claimed the largest number of patients. After the age of 16, the incidence of infection was 12.2 per cent among the females. In the male sex, 5 patients, or 0.5 percent, were seen under the age of 1. The youngest patient was an infant, 4 months old, and the oldest, a man of 61 years of age.

Mycotic diseases of the body, hands, feet and nails were mainly conditions of the middle age, but they were by no means absent in the two extremes of life. Their incidence was greatest between the ages of 16 and 30; this was true with both sexes. Among the children, their incidence was low. Under the age of 5, tinea of the hands, feet and nails was not encountered.

The incidence of favus of the scalp was highest from 6 to 15 years of age, and that of tinea of the scalp was highest between the ages of 1 and 15. In tinea of the hands, feet and nails, the preponderance of infection was in young adults.

As to the *seasonal distribution* of dermatomycoses, both favus and tinea of the scalp occurred most frequently in the spring. In tinea of the body, hands and feet, there was an abrupt rise in incidence during the summer months This was true, notwithstanding the fact that there was only very slight difference in the number of patients admitted to the skin clinic in the four seasons. In tinea of the nails, no distinct seasonal variation was observed.

Concerning the *occupation* of the patients, favus of the scalp attacked chiefly the poor. Tinea of the scalp occurred most frequently in school children and orphans. The high incidence of this disease in these two groups of patients was expected on account of their frequent exposure to the infection carried by their associates. Other mycotic infections were found widespread throughout the various social strata and occupations.

Favus of the scalp affected most frequently the vertex of the head, although the disease was also observed to attack other parts of the scalp. The affected skin of the scalp, after recovery, was usually bald, glazed, and disfigured with cicatrices.

Tinea of the scalp showed a discrete and a disseminated type. In both, small perifollicular abscesses and impetigolike lesions were not infrequently noticed. The secondary pyogenic infection often resulted in the formation of scars. Kerion celsi was very rarely met with. Out of the 1,104 cases of tinea of the scalp, only 6 patients, or 0.8 per cent, showed definite signs of Kerion.

Tinea of the glabrous skin revealed the circinate and the imbricated type. The latter form was not commonly seen. Out of the 760 cases of tinea of the glabrous skin, 7 patients, or 0.9 per cent, had lesions of this type. In these patients, the eruption invaded more or less the entire body. The involved parts were scaly and somewhat claycolored. The rough and dusky appearance was due to the presence of laminated scales arranged in concentric rings.

Tinea of hands and feet (pompholyx) revealed as a rule three main varieties just the same way as we know that from Europeon observations and in cases of the Western Hemisphere. The authors encountered the vesiculo bullous, the intertriginous and the hyperkeratotic forms. Frequently these forms occurred independently. At times, they merged into one another, so that lesions of all the three forms were present at the same time. In a great majority of cases of tinea of hands, there was an associated infection of the feet.

The vesiculo-bullous form was usually an acute disorder. It attacked the hands more frequently than the feet. They often appeared suddenly in apparently normal skin. Not infrequently, soon after their development, the vesicles or bullae became inflamed and were finally filled with pus. The intertriginous form affected only the feet. It had a predilection for the interdigital clefts and the flexor aspects of the toes. On the planter surface, the disease often spread downward beyond the roots of the toes and terminated in a well defined or irregular line opposite the head of the metatarsals. The secondary pyogenic infection not infrequently involved a large part of the feet with concurrent development of lymphangitis and lymphadenitis. The hyperkeratotic form often succeeded the vesiculo-bullous form. In some instances, the epidermis of the palms and soles was diffusely hyperkeratotic and covered with multiple layers of large scales. When the scales were detached, ruptured vesicles were often exposed.

In tinea of the nails the leucochromic type revealed an infection usually beginning at the lunula and spreading toward the free border of the nails. In the hyperplastic type, the infection usually first took place at the free border and spread toward the proximal portion of the nails.

For the mycological study as a rule, the specimens were examined microscopically and planted on the culture medium immediately or soon after collection, and *were not subjected to any procedure for the removal of the bacteria* with which they were accidentally contaminated.

The final classification of the species isolated was made in accordance with the rules laid down by SABOURAUD.

In each of the 29 patients affected with favus of the scalp, *Achorion Schoenleini* was grown. In the 664 cases of tinea of the scalp, *Trichophyton violaceum* grew in 382 instances, *Trichophyton endothrix* in 99 instances, *Microsporon ferrugineum* 92 times, *Trichophyton glabrum* 85 times. *Trichophytom cerebriforme* in 6 instances. Several variants of *Trichophyton violaceum* were obtained. They exhibited differences both in their cultural appearance and in their ability to produce pigment. Infrequently, the growth was markedly faviform, bearing resemblance to *Achorion violaceum.*

The organism provisionally designated here as *Trichophyton endothrix* did not conform in its cultural peculiarities with any of the species of the *genus Trichophyton* recorded in the mycological literature.

Microsporon ferrugineum was found to exist in two distinct varieties. In the first variety, the colonies were pale yellow in color, usually producing superficially convoluted white and short duvet (fuzz), and tending to become depigmented after being transplanted a few times. In the second variety, the colonies were much more convoluted and orange-tinged, and always remained glabrous in appearance, retaining their orange color even after cultivation for several years.

33 patients with mixed infections were observed. In four cases, *Trichophyton violaceum* was associated with *Trichophyton endo-ectothrix,* and in 21 instances the former fungus co-existed with *Microsporon ferrugineum.*

In 47 individuals with tinea infection of the glabrous skin, *Epidermophyton rubrum* was identified on 36 occasions, *Trichophyton violaceum* and *Microsporon ferrugineum* each on 4 *occasions,* and *Endodermophyton tropicale* on 3 occasions.

The fungus responsible for the disease occurring in the cruro-genital region was *Epidermophyton rubrum. Trichophyton violaceum* and *Microsporon ferrugineum* were generally grown from lesions appearing on the face, neck, and forearms. *Endodermophyton tropicale* was isolated from patients with eruptions characteristic of tinea imbricata.

In 71 cases of tinea of the hands and feet, *Epidermophyton rubrum* was isolated from 59 patients, and *Epidermophyton inguinale* from 12 patients. *Epidermophyton rubrum* was found on both hands and feet, but *Epidermophyton inguinale* was obtained only from the feet.

In 16 cases of tinea of the nails, *Epidermophyton rubrum* was grown from 15 cases, and *Epidermophyton inguinale* in 1 case.

No correlation existed between the species of fungus and the clinical manifestations of the disease.

CATANEI'S (1939*a*) extensive investigation on the *parasitic flora in Algeria according to determination of* 1000 *strains of parasitic fungi* gives us a fairly good picture of the species causing the tinea, of their ethnic origin and of their geographical distribution.

Three vast regions of Algeria were investigated: Tell, Hauts Plateaux and the Sahara. They yielded 12 species of *Trichophyton.* In cases when the causative organism was singular in the lesions, the following species were detected in order of their frequency:

Tr. glabrum Sabouraud, 1909	516 times
Tr. violaceum Bodin, 1902	406 times
Tr. acuminatum Bodin, 1902	20 times
Tr. crateriforme Sabouraud, 1902.	17 times
Tr. soudanense Joyeux, 1912.	11 times

Tr. fumatum Sabouraud, 1909	9 times
Tr. pervesi Catanei, 1937	4 times
Tr. regulare Sabouraud, 1909	1 time
Tr. cerebriforme Sabouraud, 1909.	1 time
Tr. sulfureum Fox, 1908	1 time
Tr. plicatile Sabouraud, 1909	1 time
Tr. umbilicatum Sabouraud, 1909.	1 time
	988

Of interest are the instances of fungus associations in the same lesion. The combination *Trichophyton* plus *Achorion* was found:

Tr. violaceum plus A. Schoenleini	3 times
Tr. soudanense plus A. Schoenleini.	3 times
Tr. glabrum plus A. Schoenleini	1 time

The combination of *two species of Trichophyton* occurred:

Tr. violaceum plus *Tr. soudanense*	2 times
Tr. violaceum plus *Tr. regulare*	1 time
Tr. violaceum plus *Tr. pervesi*	1 time
Tr. glabrum plus *Tr. crateriforme*	1 time

Although the parasitic flora of the tinea in Algeria comprising 13 species is variable enough, there are, however, two species, *Tr. glabrum* and *Tr. violaceum* giving the keynote to this flora due to their excessive predominance.

Surprisingly enough and in impressive contradistinction with foregoing statistics there were only European species of *Trichophyton* isolated from tinea cases in the Civic Hospital of the City of Alger by T. Brault and Viguier, and by T. Montpellier.

Tr. acuminatum	44 times
Tr. violaceum	23 times
Tr. crateriforme	8 times
Tr. plicatile	1 time
Tr. polygonum	1 time

Concerning the ethnic origin of the patients suffering from tinea infection, the author found that in the Musulman aborigines, the tinea is caused in 95 % *by Tr. glabrum and Tr. violaceum. Tr. fumatum* was a frequent parasite of the scalp in children of *Spanish* descent, while *Tr. crateriforme* was mainly found in *Italian* circles.

As to the *geographical distribution,* in the region of *Tell,* the majority of the infection (69.8 %) was due to *Tr. glabrum,* while *Tr. violaceum* was responsible for 27.7 % of the cases. Among the other species of *Trichophyton, Tr. accuminatum* and *Tr. fumatum* were the most frequently isolated. *Tr. sulfureum, Tr. regulare* and *Tr. plicatile* were found only in single instances.

On the *Hauts Plateaux* and in the region of *Aurès* only *Tr. glabrum* with predominance and *Tr. violaceum* were isolated.

In the *Sahara, Tr. violaceum* was the predominant species in all localities examined, while *Tr. glabrum* much rarer, was lacking on many places.

In the region of *Adrar* and of *In Salah, Tr. soudanense* (a species occurring in West Africa) and a new species, *Tr. pervesi* were found.

A. Catanei (1939*b*) continued in a second valuable paper his investigation on *tinea in the French colonies* including *Africa,* (Sudan, Guinée, Togo, Dahomey, Cameroon, Middle-Congo), *Madagascar* (North Tulear), *Indo-China* (Annam, Tonkin), and *French India* (Territory of Pondichery).

In 77 cases of tinea trichophytina, 65 strains were isolated in order of their frequency:

1.	*Tr. soudanense Joyeux,* 1912	31 times
2.	*Tr. violaceum Bodin,* 1902	20 times
3.	*Tr. gourvili Catanei,* 1933	5 times
4.	*Tr. fumatum Sabourand,* 1909	4 times
5.	*Tr. sulfureum Fox,* 1908	2 times
6.	*Tr. sulfureum Tr. violaceum*	1 time
7.	*Tr. rubrum Castellani,* 1909	2 times

The majority of tinea trichophytina in Africa is predominently caused by *Tr. soudanense, Tr. violaceum* not being rare in certain Africain communities, is the main causative agent in French India.

In 40 cases of *tinea microsporina* of children between the ages of 2 to 15 years, two main species of Microsporum: *M. Audouini* and *M. obesum Conant,* 1937 were isolated.

M. obesum Conant, 1937 were found 21 times [Togo (8), Dahomey (6), Cameroon (7)]; *M. Audouini Gruby,* 1843 in 14 cases [Sudan (1), Guinée (2), Togo (6), Middle-Congo (5)].

M. ferrugineum Ota, 1921 was found in Indo-China Tonkin (3) and a mixed infection of *M. Audouini* plus *Tr. soudanense* in Guinée [Conakry (2)].

In French India, the tinea trichophytina is caused by Tr. violaceum and by *Tr. sulfureum,* the former being more frequent.

In Indo-China, the tinea trichophytina of the glabrous skin is most frequently caused by *Tr. concentricum R. Blanchard,* 1895, and *by Tr. rubrum Castellani,* 1909.

Melczer (1939) gave an interesting statistics on *skin diseases in the Hungarian people* including fungus diseases. The leading fungus infection of Hungary is tinea trichophytina based on statistics of Nekám, Somogyi, Berde, Szántó, Preininger, Oláh, György, Fülöp, and of the author, comprising 540, 444 cases. In order of frequency follow: *pityriasis versicolor, tinea microsporina, favus, erythrasma* and *epidermophytia.* It is noteworthy that the tinea microsporina which based on the investigations of Neuber, Ballagi, Oláh, and others occurs mainly in childhood, was at the beginning of the twenties of this century in the southern part of Hungary unknown. It was imported from Transdanubia into the district of Szeged in the year 1927.

Szentkirályi (1939) reported on *mycological investigation* in *school children of the district of Csongrád, Hungary.*

The investigations were carried out by cooperation of the Skin Clinic of the University Szeged, the Institute of Hygiene in Hodmezövásárhely. In the year 1937/38, 20,520 school children were examined, showing a frequency of fungus diseases of 0.56 % (tinea microsporina 0.5 %, tinea trichophytina 0.05 %, and favus 0.018 %).

Liebner (1939) gives the result of his investigation of *fungus infection in connection with eczema of the infancy and the childhood.* In these eczemas, in cases quite arbitrarily picked out among others, fungus infection could be detected in 60 %. By cultural examination of their flora the following species were isolated, the clinical picture of the cases being otherwise identical; species of *Oidium, Epidermophyton, Trichophyton, Common molds: Aspergillus, Mucor, Penicillium.* Whether in these cases the fungus infection is primary or the fungi are only secondary invaders on the floor of the primary eczema, the author could not give any definite answer at the present moment.

Catanei (1939*c*) further investigated the *tinea of the scalp in the French*

Colonies, altogether 129 cases. He found culturally 77 cases of *Trichophyton* infection, 40 cases of *Microsporum* and one *Favus*. The material (hair, scales), was sent to the author at times from great distances revealing the great resistence of these infecting agents in spite of high temperature, dryness, etc. The cultures, however, yielded in most of the specimens positive results. The tinea trichophytina was mainly caused in West and Equatorial Africa by *Trichophyton soudanense*, on the second place by *Tr. violaceum*. In a few cases a new species of *Trichophyton*, the *Tr. gourvili* was found. Tinea microsporina had as causative organism ordinarily *Microsporum obesum*, occuring more frequently than *M. Audonini*. In French India the most frequent species are *Tr. violaceum* and *Tr. sulfureum*.

IRGER, KOWALSKY and LIBERMAN (1939) reported on treatment and prophylaxis of fungus diseases acording to the records of the mycological center of the Lenin district of Moscow and of the Skin Clinic of the 2nd Moscow Medical Institute. The activity of this mycological center is based upon dispensary methods (outpatient department). The registration, almost without any gap, of the mycotic diseases, the disclosure of all the sources of infection, and their elimination were successfully carried out due to the thorough investigations in family, school, kindergarten, in cooperation with school physicians and pediatricians under the assistance of a properly trained nursing staff. A permanent control of the results secured the success. Among 1766 sick, there were only 10.7 % adults (over 18 years of age), 58.5 % children under 8 years of age, mainly in kindergarten age, and 30.5 % school children; there were more boys than girls. Among children the freqeunt form of the fungus disease was that of the scalp, at times with involvement of the glabrous skin and the nails. The involvement of the glabrous skin alone was not frequent and isolated onychomycosis was an exception. Among adults, women outnumbered the other sex. Almost one third of the infections among adults was detected by the examination of the families. In these cases one found very often insignificant changes, difficult to be diagnosed, like hair stumps in the form of "black points", without reddness and scaling, particularly on the occiput. Diseases of the nails occurred in 30 %. Fungus diseases of the adults, often unnoticeable, persistent for years, stubborn, play a most importent role in the spreading of the infection. Sources of infections of other kind, like domestic animals, utensils, clothing, barbers' shop made up only 9 % of the grand total. Among the fungus species in order of their frequency: *Trichophyton* was represented in 75.8 % (mostly *Tr. violaceum*, less *Tr. crateriforme*, seldom *Tr. gypseum*), *Microsporum lanosum* 14 %, *Achorion schoenleini* 7.9 %.

As to the therapy, Thallium aceticum was used in children up to three years of age, administered in the usual dose, 8 mg per kg body weight, without any untoward effects. In children between 1 to 12 years of age showing a limited number of foci on the glabrous skin (1—3), the method of the local application of Thallium aceticum was successful according to LIBERMAN. In the greater part of the children, as well as in adults, X-ray irradiation was carried out. Fungus infections of the glabrous skin were much more stubborn in adults than in children and withstood at times even energetic anti parasitic therapy. In the experience of the authors, onychomycosis was best treated by means of the method of PELLIZARI, a 50 % pyrogallic acid-salve applied on the attacked nails.

GOHAR (1939) gave a *record of some fungi isolated* in Egypt, discussing the very timely question of *true mycosis vs. pseudo-mycosis*. Because of the multiplicity of saprophytic fungi existing in nature, it is arbitrary to label all cases from which fungi are isolated as true mycosis. A vast number of species are ingested with food (e.g. yeasts and yeastlike bodies on grapes, mold spores on many other fruits, vegetables) and drinks (e.g. beer, wine), gain admittance by inhalation to the respiratory apparatus (divers spores of molds in the air), enter the body on penetrating foreign bodies (injuries), or are merely deposited from the air or by contact on natural openings or some breach of surface and are found on isolation, to represent a rich variety of nearly all classes.

We agree very much with the author who emphasized that arguments, such as the pathological material having been especially collected, need not detract from the value of a sceptical attitude that should be often adopted when some new species, claimed to be pathogenic to man or animal, is reported.

To illustrate his point, the author discusses the mycotic flora of bronchomycosis, tinea cruris, gluteal blastomycosis of the type KARTULIS, chromoblastomycosis and sprue.

In order to determine the incidence of *fungi in the sputum*, one hundred specimens were selected haphazard from among those patients sent to be examined for *Mycobacterium tuberculosis*. Only cultures were used on SABOURAUD's medium.

Whether Mycobacterium was found positive or not, whether the clinical tentative diagnosis was tuberculosis of the lungs, or other various lung affections, mostly bronchitis and pleural effusions, a few pulmonary abscesses, pneumonias, the following species were isolated: *Aspergillus fumigatus*, *Aspergillus niger*, *Syringospora albicans* (*Robin*, 1853) *Dodge*, 1936, *Mucor racemosus*, *Rhizopus niger*, *Redaellia elegans Ciferri*, 1930, *Torulopsis nitritophila Ashford and Ciferri*, 1930, *Absidia corimbifera*, *Poeciliomyces burci*, (Pollacci, 1921) Thom 1930, *Acremoniella atra Corda*, 1937, *Cephalosporium acremonium Corda*, 1839, *Penicillium sp.*, *Alternaria sp.*, *Pullularia pullulans* (Bary, 1868) *Berkhout*, 1923, *Geotrichum sp.*, *Saccharomyces blanchardi Guiart*, 1906, *Hansenula anomala* (Hansen, 1891) Sydow, 1919.

Five cases of *Tinea cruris* unexpectedly supplied cultures of one of the particular species isolated so far from Tinea imbricata, namely *Endodermophyton concentricum*, although *T. cruris* and *T. imbricata* are among the few infections, like favus, where it is considered superfluous to make cultures from the lesions because of the uniformly undeviating results.

One case of a new and rare disease in Egypt, ALI IBRAHIM's disease, different from, but suggestive of, KARTULIS' blastomycosis, yielded *Fusarium solani*. Three cases of gluteal fistulae and sinuses conforming to KARTULIS' description of blastomycosis of that region, failed to furnish the specific yeast of the disease.

Two cases of *chromoblastomycosis* were examined. *Phialophora verrucosa*, a recognized etiological agent of that disease, was obtained from one case, and *Pullularia pullulans*, a common nonpathogenic saprophyte, was grown from the second.

Cultures made post mortem from the large *intestine and tongue* of a case of *sprue*, supplied colonies of Syringospora psilosis illustrating the fallacy of a long-established theory that sprue has for a specific etiological agent this fungus. Today we know that sprue is a avitaminosis.

One of the most exhaustive and highly important statistics on dermatomycoses was contributed by PERPIGNANO (1939) dealing with the Italian province of *Cagliari*. This painstaking piece of research is in the same time an excellent contribution to the geography of the dermatomyces even though it is concerned only with one single province. The whole concept of the investigation carried out with consistence, in the most systematic manner, could be and should be an example to emulate others to work up the mycotic flora of smaller or larger geographical units. This type of research is called to fill in large gaps still existent in our knowledge concerning the occurrence, geographical distribution, fluctuations and epidemiology of dermatomycoses and their causative organisms.

PERPIGNANO's statistics is based on observation of six years, from 1933 to 1938. It comprises 2107 cases of dermatomycoses, about 350 cases per annum, in a territory covering 9298 square Km, inhabited by 507, 203 people.

One of the dermatomycoses, *tinea microsporina*, was not found in *Cagliari* at present, although it was reported in Sassari by PELAGATTI in 1909 and by MANCA PASTORINO in 1933.

The following table shows the localization of *tinea trichophytina* and *tinea favosa*:

Year	Tinea trichoph. capitis	Kerion trichoph.	Tinea trichoph. cutis	Onychomycosis trichoph.	Sycosis trichoph.	Tricophytid	Favus	X-ray treatment of children
1933	251	3	46	2	2	1	53	279
1934	373	4	39	3	—	2	51	333
1935	252	2	43	2	3	2	11	185
1936	235	8	46	—	4	1	15	178
1937	227	4	56	1	5	2	18	175
1938	262	6	52	5	—	1	14	218
Totaal	1600	27	282	13	14	9	162	1368

The following *Trichophyton* species were isolated:

1.	*Trichophyton*	*violaceum*	63.15 %
2.	,,	*rosaceum*	4.1 %
3.	,,	*exicatum*	0.2 %
4.	,,	*crateriforme*	7.61 %
5.	,,	*acuminatum*	3 %
6.	,,	*gypseum asteroides*	0.8 %
7.	,,	*plicatile*	15.3 %
8.	,,	*farinulentum*	0.5 %
9.	,,	*glabrum*	4 %
10.	,,	*cerebriforme*	1 %
11.	,,	*niveum radians*	0.05 %
12.	,,	*album*	0.05 %
13.	,,	*granulosum*	0.05 %

The relation of the different species of dermatomyces to the type of the clinical lesion can be evaluated from the subsequent tabulation:

			%
Tinea trichophytina of the scalp	Torpid	*violaceum*	48.1
		glabrum	4
		plicatile	15
		acuminatum	2.05
		crateriforme	2.5
		exicatum	0.2
		gypseum asteroides	0.4
		gypseum farinulentum	0.5
		granulosum	0.05
		rosaceum	3.55
		cerebriforme	0.5
	Inflammatory	*violaceum*	4.15
		crateriforme	0.2
		acuminatum	0.15
		plicatile	0.3
		cerebriforme	0.5
		rosaceum	0.55
		niveum radians	0.05
		asteroides	0.2
	Kerion	*violaceum*	0.75
		acuminatum	0.10
		crateriforme	0.5
Tinea trichopytina of the beard	torpid	*violaceum*	0.35
		crateriforme	0.05
		acuminatum	0.05
	sycosis	*violaceum*	0.15
		crateriforme	0.05
		acuminatum	0.05
Tinea trichophytina of the glabrous skin	torpid	*violaceum*	5.7
		crateriforme	2.15
		acuminatum	0.55
		trichophyton album	0.05
	vesiculopustular	*gypseum*	0.2
		violaceum	3.35
		crateriforme	1.55
		acuminatum	0.05
	Onychomycosis trichophytina	*violaceum*	0.6
		crateriforme	0.05

The computed statistics of dermatomycoses for the provinces *Sassari, Nuoro* and *Cagliari* concerning tinea favosa, tinea microsporina and tinea trichophytina is shown in the following table:

	Province of Sassari			Province of Nuoro	Province of Cagliari			
	Pelagatti-Barbaglia-Manca Pastorino			Rivelloni	Serra-Bernucci-Pinetti			Perpignano
	1909	1914	1933	1934	1922	1923	1933	1937
Tinea favosa:								
Ach. Schoenleini	—	—	2,41	11	29	—	6,7	8,3
Tinea microsporina:								
M. lanosum	—	—	6,33	—	—	—	—	—
M. Audouini	—	—	3,61	—	—	—	—	—
M. tomentosum	—1)	—	2,41	—	—	—	—	—
Tinea trichophytina:								
Tr. Rosaceum	—	2,85	3,01	1,66	5,7	1,5	1,7	4,1
Tr. Exicatum	—	—	0,03	0,83	—	—	2,5	0,2
Tr. Crateriforme	—	—	4,81	—	—	36	2,7	7,61
Tr. Acuminatum	—	7,62	2,02	8	11	2,5	3,6	3
Tr. Gypsem asteroides	—	—	1,02	—	5,3	2	—	0,8
Tr. Effractum	—	—	—	—	—	—	3,6	—
Tr. Paracrateriforme	—	—	—	—	—	—	17,3	—
Tr. Plicatile	—	—	—	4,56	—	—	4,2	15,3
Tr. Farinulentum	—	—	—	—	—	—	0,8	0,5
Tr. Granulosum	—	—	—	—	—	—	—	0,05
Tr. Umbilicatum	—	—	—	—	—	2	—	—
Tr. Niveum	—	—	—	—	—	0,3	—	0,05
Tr. Violaceum	—	43,81	50,62	85	38,3	55	61	63,15
Tr. Glabrum	—	23,81	1,02	—	17,7	—	—	4
Tr. Cerebriforme	—	21,90	2,71	—	20,8	0,7	3,6	1
Tr. Album	—	—	—	—	—	—	—	0,05

Trichophytid was observed in nine children, from 7 to 13 years of age, 0.53 % of the sum total. In these cases the primary lesion was always on the scalp. In six cases the culture yielded *Tr. violaceum*, in two *Tr. plicatile*, and in one *Tr. cerbriforme*. It is interesting that the trichophytid was always elicited by some irritation: spontaneous (kerion, impetigo), chemical (salves, oleum crotoni), or physical (X-ray). Blood culture was tried in all cases repeatedly, but only *Tr. Violaceum* was recovered in only two instances.

(c) *Culture*

ALDO CASTELLANI (1939) gave brief notes on three new species of Trichophyton: *Tr. batonrougei, Tr. guzzonii, Tr. tenuishypha* and a little known species; *Tr. louisianicum*. The investigations as to the taxonomic position of these fungi is not yet as complete as necessary and the exact taxonomic position and their differentiation from other dermatophytes will be more

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1) isolated four times in 189 cases of tinea trichophytina.

completely reexamined. For the time being they are all placed in the genus Trichophyton.

(*a*) *Trichophyton batonrougei* n. sp. was isolated from four cases of Tinea capitis in Negro children in New Orleans. The fungus is fairly easily grown from the hair and the scales, but it is usually quite scanty on microscopical examination in the hair and scales, so much so that it is difficult to say yet with absolute certainty whether it is endothrix or ectothrix or neoendothrix: it appears to be endothrix.

The giant colony presents, especially in its central portion, a peculiar brownish color comparable with the color of a mature chestnut. The fungus becomes pleomorphic fairly rapidly and the color is frequently lost in the subcultures. The microscopical examination shows the presence of abundant mycelium which may be of varied type, including the racquet type. Closterospores (fuseaux) have not yet been found and definite conidia or aleurospores are extremely scanty. In hanging-drop peptone water cultures at 30° C, large terminal somewhat fusiform, occasionally almost triangular terminal structures, are seen, and also somewhat similar intercalary structures. It is interesting to note, however, that in hanging-drop cultures of two of the examined strains bodies of various type and size and appearance have developed inside the structures. Whether they are spores, analogous perhaps to ascospores, further research can only decide. In hanging-drop cultures the width of the mycelial hyphae varies between 2 and 4 μ, most of them being about 2.8 μ. The large terminal fusiform structures measure 16—28 by 24—36 μ. The peculiar sporiform bodies inside such structures may be of various sizes, most of them 2 to 4 μ. Biochemical characters are thoroughly given. Attempts at inoculating the fugus in guinea-pig and rabbits had failed.

(*b*) *Trichophyton guzzonii, n. sp.*

This fungus was isolated from a case of ringworm of the dorsum of the foot in a Negro boy in New Orleans. The fungus grew abundantly on all the ordinary laboratory media. On glucose agar the colony, when fully developed, is of large size, somewhat irregular, covered with fairly abundant rather short duvet. Some portions of the growth are orange in color, others reddish or rust colored and others whitish. The appearane of the culture is somewhat similar to *Trichophyton dankaliense* CASTELLANI (J. Trop. Med. & Hyg., December 15, 1937). The microscopical examination of hanging-drop cultures shows the presence of abundant mycelium. Some hyphae are delicate, 1 to 2 μ in diameter, others measure 6 and 12 μ. Definite aleurospores have not been seen. Typical chlamidospores not yet noted. Closterospores absent. Biochemical characters are given. The cultures on glucose agar closely resemble those of *Tr. dankaliense* case, but the microscopical examination shows the two fungi to differ, as in *Tr. guzzonii*, the peculiar clusters of spores found in *Tr. dankaliense* are never noted. *Tr. ferrugineum* Ota attacks the scalp and its cultures on glucose agar have a more uniform rusty color. Experimental inoculation in rabbits and guinea pigs have not succeeded.

(*c*) *Trichophyton tenuishypha n. sp.*

This fungus has been isolated from a European women in London, who lived in the West Indies some years ago. Colony on glucose agar is of a white color, fluffy from the very beginning. After 3 or 4 weeks, portions of the duvet, expecially at the periphery, and portions of the colony itself became black, and later the whole of the growth may become black and the medium may take the same color. The characteristic feature of this fungus is the thinness of most of its mycelial hyphae, especially in young cultures. They are usually between 1.2 and 2.5 μ. In the central portion of the cultures the mycelial filaments form a sort of prosenchyma. No closterospores and no definite aleurospores have been found so far. Biochemical characters are given. The black pigmentation of the aerial mycelium (duvet) and the thinness of the hyphae are differentiating it from other Trichophyton. Animal inoculation failed so far.

(*d*) *Trichophyton Louisianicum Castellani,* 1927 was found in New Orleans in several cases of Tinea capitis in Negro children. (New Orlenas Med. & Surg. J. 79 : 896, 1927). The fungus is very scanty in the hair and it cannot yet be said with certainty whether it is an endothrix, neoendothrix, or ectothrix; in all probability it is an endothrix. On glucose agar 4 per cent of the growth consists at first of a minute, nodule with radiating, mostly submerged, hyphae of a yellowish, often canary yellow, color. Three or four weeks after inoculation the colony is large, usually with a central white portion of white longish duvet springing up from a rather hard mass. The peripheral portion is yellowish or there is a margin of reddish or brownish color. Pleomorphism is frequent. Closterospores are present. Definite aleurospores have not yet been seen. In hanging-drop culture, mycelial threads vary between 3 and 6 μ in diameter. Closterospores are 72—80 μ. Biochemical characters are given.

It has been suggested by DODGE that this fungus may be an American strain of *Trichophyton soudanense* Joyeux. It would appear, however, that the fungus described by Joyeux has somewhat different characters. Its colony has been described as a small apricot yellow peak which grows and becomes irregularly folded and about the ninth and tenth day the base expands into a white mat with radial folds. In *Trichophyton soudanense* aleurospores are present in either simple or compound thyrses and closterospores are absent, while in *Trichophyton louisianicum* aleurospores are absent and closterospores present. Experimental inoculation in guinea pig is negative.

CASTELLANI believed at one time that the fungus was close to *Tr. sulphureum* Fox, but the colony of *Tr. sulphureum* is crateriform and has a speckled appearance. *Tr. ochraceum* Sabouraud and *Tr. flavum* Bodin have cerebriform colonies. The color of the glucose agar cultures of *Tr. louisianicum* may closely resemble the canary-yellow color of the glucose agar cultures of *Epidermophyton floccosum* Harz (= cruris = inquinale), but *E. floccosum* produces closterospores of somewhat different shape without the minute tubercles and does not attack the scalp.

ERIKSON (1940) *presented studies on some lake-mud strains of micromonospora.*

Preparation of stained paraffin sections. A review of the work of earlier investigators of colonial organization of bacteria and other micro-organisms has been given by GREENE (1938). The technique recommended by him and earlier by HUTCHINSON (1907), of covering the colonies with lukewarm agar to preserve the surface intact, was employed. It was later found that with the firm compact Micromonospora colonies this complete embedding was not necessary, provided care was taken in handling the specimen. Colonies of varying ages from 3 days to 5 months were removed with a portion of the agar in which they had grown, and transferred to the fixing agent. This and subsequent liquids were pipetted in and out of the phial in order to avoid disturbance of the colony. The standard meduim for growth was CZAPEK's sucrose agar. Bottom and surface colonies from liquid CZAPEK's sucrose media were also prepared for sectioning.

Acetic acid-formalin-alcohol, BOUIN's, and KARPECHENKO's fluids proved equally good as fixatives. The most satisfactory dehydration was obtained by means of the well known alcohol, cedar-oil technique. Paraffin sections were cut with a rotary microtome at a thickness of 5 micra. Differential staining was effected by means of MALLORY's and FLEMMING's triple stains, by iron-hematoxylin counterstained with safranin or orange G, and by erythrosin counterstained with GRUBLER's ,,Nachtblau''. In all cases the spores took an intense stain and were readily distinguished from the vegetative mycelium. About 150 sections were cut.

Examination of sections. The most interesting fact which emerged from a study of these sections was the appearance of a well-marked zonation of spore-bearing hyphae within the colony. At direct microscopic observation of living material it was noted that sporulation generally takes place in the centre of the colony on about the second day, and that the growth then becomes heaped up — or, alternatively, develops into the medium — thus impeding vision, while it is not until about the second week or later that the glistening superficial spore layer, which is usually associated with dark pigmentation, is produced. The series of vertical sections of colonies of different ages would, it was hoped, reveal whether there was any organized structure within the colony resulting in this production of a superficial spore layer.

Ten strains of the species-group *Micromonospora chalceae* (Foulerton) Orskov, derived from lake mud or in one instance from lake water, have been examined in detail. Physiologically, they have been found capable of growing on a very large variety of more or less resistant organic compounds, being especially active in the decomposition of chitin, cellulose, and, to a lesser extent, lignin. Morphologically, they have been shown, by means of thin sections, to possess a definite colonial organization characterized by the zonation of sporulating material. Their possible role in lacustrine ecology has been discussed.

AREA LEAO (1940) *reviewed the genus ,,Trichosporon'' Behrend,* 1890, *giving the description of a new species, ,,Trichosporon minor ''n. sp.*

He studied the Trichosporon genus from the standpoint of systematics and as the producer of the white or trichosporic piedra as well as of generalized internal mycoses of the gummatous type. He established the priority of the term Trichosporon given by BEHREND. He studied the distinction between the two piedras: the white or trichosporic piedra, and the black or asterinic piedra. He then studied the changes produced by the two fungi on the hair attacked by the parasites and considered these changes as being an indispensable factor for the fixation of the parasite on the hair and for the further formation of the nodule, this phenomenon though not constituting a characteristic feature for the separation of the species. At last, he reported on a new species of piedra, piedra axillaris, produced by *Trichosporon minor n. sp.*, giving the following description of it:

Trichosporon minor n. sp. — a fungus producing piedra axillaris, forming on the hair of this region small nodules of grayish color, of hard consistence and variable in size, constituted by roundish, elliptical or oblong cells, measuring 0,5 to 4 micra in diameter, some of them gemmulating, and forming chains of two to four elements. It causes the separation of the hair cuticle upon which the parasite fastens, yet without penetrating into the deeper layers of the hair. In artificial culture media, it forms colonies initially flat, of moist and velvety surface, trimmed with thick and salient fringes which cross the colony through two diameters, or white flat colonies resembling Actinomyces culture, or vermicular colonies. It ferments no sugars and dissolves gelatin tardily.

MACKINNON (1940) *made an important contribution to the epidemiology of Autochtonous Dermatomycoses in Uruguay.*

Three foci of favus were registered with nine cases. Studies were carried out in tineas in horse and guinea pig as sources of human infections. The greater part of tinea microsporina is of animal origin with the causative organism of *Microsporum canis*, Bodin. Generalized epidermomycosis and onychia were caused by *Trichophyton Rubrum* (Castellani). Trichomycosis Nodularis of the scalp („Piedra americana") was recently found in SALTO, PAYSANDU and in RIVERA in which localities this condition deems to be fairly frequent. Clinical, bacteriologic and mycologic studies were carried out in intertrigines caused by yeastlike organisms. Experimental investigations about the pathogenicity of yeastlike organisms revealed that those organisms showed the greatest virulence which were isolated from intertrigoes of DUBREUILH and JOULIA type. Strains which were not the etiologic agents did not show any virulence in experiments. Studies in dissociation and variation of pathogenic yeasts revealed that it was almost always the *Candida* (Mycotorula) *albicans*. Pityriasis vericolor showed an incidence of 0,5 % among the patients in the outpatients clinic. New clinical, mycological and pathological investigations were carried out in onychia and paronychia. Only one case of chromoblastomycosis was observed. On the other hand. 21 cases of sporotrichosis were diagnosed mycologically, an incidence revealing the realtive frequency of this disease in rural areas. In several cases of sporotrichosis the „asteroid form" (Splendore) of the fungus was observed. In one case of maduromycosis with red granules a new species of the causative fungus was discovered: *Rubromadurella Langeroni*, Talice, 1935.

A useful bibliography of Uruguayan dermatomycological publications (61 references) is given.

MOORE (1941) *reported on the use of the chorioallantoic membrane of the developing chick as a medium for the cultivation and histopathologic study of pathogenic fungi.*

Technic. As employed here, the method is essentially the same as that used by GOODPASTURE and BUDDINGH and by BURNET for cultivating viruses in the chorio-allantoic membrane of the chick embryo. The coverslip method was employed in preference to the shell-flap method. The former is best adapted for the study of developing lesions.

Because of the slowness of development of fungi, it was found advisable to use eggs incubated 10 to 14 days, as advocated by GOODPASTURE, depending on the type of organism to be used. Yeastlike organisms show a development time of approximately 5 to 7 days, whereas filamentous forms have a longer evolution time, varying from 5 to 11 days. For such, therefore, it was necessary to use eggs incubated only 10 days.

After inoculation, the eggs were placed in a regular bacteriologic incubator and maintained at a constant temperature without turning. For fungi it was found best to maintain the temperature at approximately 33° C. The eggs were examined daily and, when growth had developed on the membrane for a sufficient period, the shell was cut just below the surface of the chorio-allantois and removed. A small amount of ZENKER's fixative (with 5 per cent glacial acetic acid) was dropped on the inoculated area to harden the membrane, which was then removed with a pair of fine scissors with curved points and placed in ZENKER's solution. The membranes were then embedded in paraffin, sectioned and stained with LOEFFLER's methylene blue and eosin, cleared in xylol and mounted on slides.

Materials. The organisms cultured and the diseases which they produce are listed as follows in the order of presentation:

I. Superficial dermatomycoses (superficial desquamation, pityriasis)
  A. *Malassezia furfur* (pityriasis or tinea versicolor)
  B. *Pityrosporum ovale* (seborrheic dermatitis)

II. Dermatomycoses (epidermic and dermic lesions)
  A. *Trichophyton gypseum* (trichophytosis, tinea, ringworm)
  B. *Epidermophyton inguinale* (epidermophytosis, athlete's foot, tinea cruris)
  C. *Achorion Schoenleini* (favus)
  D. *Microsporum canis* (microsporosis)

III. Cutaneous, subcutaneous, mucous membrane and internal organ involvement (localized cutaneous lesions, granulomata, deep-seated ulcerative lesions, mucous membrance plaques, lymph stream invasion with dermic and subsequent epidermic involvement, and visceral or generalized diseases)
  A. *Monilia albicans* (moniliasis)
  B. *Geotrichum versiforme* (geotrichosis, mucous membrane and bronchial lesions)
  C. *Zymonema dermatitidis* (blastomycosis)
  D. *Cryptococcus hominis* (cryptococcosis, torulosis)
  E. *Coccidioides immitis* (coccidioidal granuloma)
  F. *Sporotrichum Schencki* (sporotrichosis)
  G. *Actinomyces bicolor* (actinomycosis)
  H. *Monosporium apiospermum* (maduromycosis, Madura foot)
  I. *Phialophora verrucosa* (chromomycosis)

When possible, young subcultures of several days' growth were used in order to obtain active proliferation. Several of the organisms had been isolated from human lesions only a short time prior to experimentation.

A number of fungi representative of the causative agents of various types of lesions which develop in man were successfully inoculated in the chorio-allantoic membrane of the developing chick to determine the effect on the fungi and the reactivity of the chorio-allantois to the parasites. Fertilized eggs that were incubated 10 to 14 days were used in this study. Inoculations were made directly on the chorio-allantoic membrane. Macroscopically, the lesions manifested themselves as thick or thin, white, grayish or grayish yellow to light brown, confluent of discrete plaques on the membrane, depending on the type of organism used.

Microscopically, the membrane reacted in the form of nodules, ulcers, superficial growths and hyperplastic lesions showing varying degrees of proliferation. There was increased activity in most cases evidenced by intense infiltration of ectodermal cells, red blood cells, fibroblasts, leukocytes and monocytes, with inflammatory changes in the mesoderm and ectoderm, and in some cases in the entoderm, and marked edema at the sites of fungous growth. This resulted in a thickening of the membrane which was increased also by the invasiveness, in some cases, of the fungous elements.

The fungi grew luxuriantly on the chorio-allantois, were easily demonstrated with methylene blue and eosin, and in most cases showed a reversion to their parasitic morphology in from 5 to 11 days. Strangely enough, with yeastlike organisms this reversion may take place throughout the entire growth or, as is the case with some of the filamentous forms, at the periphery of the fungous inoculum, in the ectodermal layer or at the surface of the ectoderm where the amount of mycelium is relatively small.

By this method it has been possible to develop lesions, some of which have hitherto required human subjects. The value of the chorio-allantois for this purpose may be emphasized also because the cost is much less than if experimental laboratory animals are used and the time necessary for the development of diagnostic features frequently is much shorter. The gratifying results which have been obtained indicate great possibilities in investigative work with fungi and thus warrant the continued use of this method in mycology.

(*d*) *Trichophyton*

(1) Clinic

M. Ota (1939) reported on Palau-Tamushi Takasugi or Ponape-Tamushi Takasaki, a peculiar *dermatomycosis*, occuring on South Sea Islands, particulary on Palau and Ponape, described first by Takasugi as Palau-Tamushi (Tamushi means tinea), later named by Takasaki as Ponape Tamushi. Fuji advanced the opinion that this disease is nothing but a kind of trichophytosis, caused by Epidermophyton rubrum Castellani (*Trichophyton purpureum* Bang). The author agrees with Fuji having been succeeded in isolating the same fungus from scales of a patient sent to him by Iseki from Palau Islands. The difference in the clinical picture between eczema marginatum and Palau-Tamushi depends probably not on the biological properties of the causative organism, but much more on climate and race.

W. Harding Kneedler investigated the *tinea nodosa of the scalp hair in school children in South Siam* (*Chieng Mai*). Among 1,120 school children, 311 (27.9 %) were found to have tinea nodosa of the scalp hair. The disease in Siam is comparatively uncommon among adults.

The relative incidence of the disease by grades in school is shown from the observation of 457 girls in two Siamese schools in Trang. This is as follows:

Grade	1	2	3	4	5	6	7	8	9
Percentage	30.9	41.7	45.0	43.2	30.6	38.4	35.1	20.0	14.3

The ages of all children found diseased (without reference to the total numbers of children of each age examined) was as follows:

years	5—7	8—10	11—13	14—16	17—19
numbers	39	100	102	68	7

It is regrettable that the mycology of the cases is disposed of with the short remark that the fungi did not invade the hair shaft. There was no relation in incidence between tinea nodosa and pediculosis. The use of oil on the hair does not seem to be an important factor in the incidence of the disease. The most effective treatment next to shaving the scalp is washing with red mercuric iodide soap; a satisfactory solvent for the nodules has not been found.

Gougerot, Blum and Duché, observed a case of an erythemato-squamous, eczematiform, diffuse tinea trichophytina, clinically all the more interesting because it appeared under the picture of a generalized eczema all over the body. Mycologically it was of interest the presence of Trichophyton gypseum which was found not only in plantar lesions but also in lesions of the thighs, legs, and arms. The clinical picture hardly allowed to think in advance of a epidermomycosis. Cure by iodine-alcohol-paint.

Catanei *and* Grenierboley *observed tinea trichophytina of the glabrous skin* in Tonkin, six at Hanoi and one at Hoa Binh. They succeeded isolating in four cases the causative organisms: *Trichophyton concentricum* R. Blanchard, 1895 and *Trichophyton rubrum* Castellani, 1909. each one in two cases respectively. The fungus disease due to *Tr. concentricum* is clearly individualized by its clinical characteristics and it is well known in the Far East as tokelaw.

M. Ota and S. Kawatsure first demonstrated the inoculability of *Tr. concentricum* in guinea pigs. The authors studied its pathogenicity for the guinea pig and the monkey succeeding ordinarily in their experiment.

The inoculation of *Tr. concentricum,* by means of a culture, in the guinea pig causes, from the tenth day, small erythematous patches progressively increasing in size. In a week thereafter the tinea is characterized by small erythematosquamous plaques round, from 3 to 5 mm in diameter, with clear cut edges slightly elevated. The surface of these lesions consists of more or less regular concentric circles. This characteristic, never mentioned before, recalls the clinical aspects of the human lesion. In the course of the third week after the inoculation the primary lesions coalesce resulting in an irregular plaque which still remains clearly limited. Spontaneous healing occurs as a rule within six weeks. From the clinical point of view, the experimental tinea in guinea pig with *Tr concentricum* is quite different from any other experimental tinea in this animal due to its peculiar clinical aspect and its long duration. Scales taken from the lesions after the second week are flooded with irregular septate mycelium.

The same experimental tinea in the monkey (M. Inuus) lasted two months and a half. The invasion of the epidermis by the *Tr. concentricum* was very marked, the hair, however, never was attacked.

Retro-cultures were constantly carried out, the importance of which is duly emphasized.

The pathogenicty of the *Trichophyton rubrum* Castellani, 1909 was studied in the guinea pig and in the monkey. Inoculation of cultural material caused an invasion of the hair in accordance with the type ectothrix megaspore.

Minami and Hara described a case of *sycosis trichophytica.* A 29 year old man suffered recurrently from a mycosis of the genitocrural region resembling eczema marginatum. Within two months, two large abscesses developed on the right cheek, starting with red nodules on the chin transformed later in a tumor-like mass. The tumors were soft and bald and under pressure they discharged pus out of the follicles. There was 10 % eosinophilia in the blood. The culture yielded *Sabouraudites asteriodes* (gypseum group). Complement fixation reaction as well as the intracutan test with the fungal vaccine were positive. Histologically there were a perifolliculitis and cutan abscess present. The lumen of the follicle were distended and filled with hyperkeratotic debris intermingled with mycelia. In the depth of the cutis there were a few degenerated follicles with hyperkeratotic debris and fungal elements, surrounded by several foreign body giant cells. The patient was cured by wet dressings with rivanol, X-ray, tar, tincture of iodine, and trichophytin injection.

Karrenberg observed a case of *sycosis parasitaria profunda.* A 37 year old soldier had nodular lesions in the nape of the neck for half a year. Treatment with salves was without success, after X-ray irradiation only little improvement. From the neck the condition spread to the right mandibular region forming big nodes partly with a papillomatous surface accompanied by a severe inflammatory reaction and lymphadenitis. Fungus elements (ectothrix) were positive in the hair. On admission he showed over the whole trunk a disseminated papular exanthem: lichen trichophyticus. Trichophytin reaction: ++++. The trichophyton injection accentuated the trichophytid. Trichphytin, unspecific protein therapy and wet dressings improved the condition. It is pointed out, and we can confirm this not unimportant therapeutical remark, that deep seated trichophytosis (sycosis, kerion) shows exacerbation after treatment with salves.

FIGUEROA *and* CONANT *reported the first case of tinea imbricata caused by trichophyton concentricum blanchard* 1896, *from Guatemala.*

The first case of tinea imbricata in Guatemala is described. *Trichophyton concentricum* Blanchard 1896 was isolated from this case. A discussion of the literature concerning fungi isolated from other cases revealed that *E. tropicale, E. indicum, E. Castellani, E. Mansoni,* and *E. Roquettei* should be reduced to synonymy with E. concentricum. The Endodermophytons should be placed in the genus Trichophyton and the genus Endodermophyton dropped.

LUBCHENCO *gave an excellent and detailed report with fine illustrations on mycotic infection in Northeastern Colorado.*

In northeastern Colorado several cases of *Trichophyton Ectothrix Microides* have been seen, three of which were so typical that their description will, to a certain extent, embrace the characteristics of the location, the lesions, and the fungus itself. The appearances of these cases in their chronological order gave the gradual development of the parasite in the human skin. One of these cases dealt with infection of the wrist of mild character, the second, of an infection of the right temple with acute and very pronounced inflammatory reaction on the skin, and the third, one of the left temple, again dealing with a mild infection.

The character of the fungus itself was the same in these cases. It was profuse. In the scales the fungus appeared as a mycelium with rectangular elements and a filamentous mycelia, as small spores in chain and groups, and as disseminated singular and grouped spores of various sizes from 2 to 6 micra. The presence of broken hairs was not manifested as much as in microspora. The fungus of the sheath and the hair was profuse.

These cases proved that time is an important factor in these diseases — short time prevents the development of more pronounced inflammatory reaction in the skin and also prevents a greater suppuration, bogginess and infiltration, which in their turn produce the pustulosis and keratosis.

The species of *Trichophyton Ectothrix Microides* and the genus *Microsporon* have two factors in common: a sheath on the hair and small spores of the sheath, by which these two fungi may be mistaken one for the other. To avoid the possible mistake, one may consult below the comparative table of these fungi.

Comparative table

Tr. Ectothrix Microides	*Microsporon*
1. Lesions of Skin	
a. Inflammatory, suppurative, pustular and boggy lesion.	*a.* Marginated circular spots with dry grayish scales.
b. Typical kerion formation.	*b.* There is no redness or suppuration. Inflammation and kerion occasionally in animal type.
c. Elevated.	
2. Hairs	
a. Unbroken, pulled with resistance. Broken ones met occasionally.	*a.* Broken. Uniform stumps of 2—4 mm. protrude from skin, pulled without resistance.
3. Sheath	
a. Coat of spores and fragments of mycelia.	*a.* Coat of spores only.
b. No special characteristic.	*b.* Frosted appearance is a characteristic.

4. Fungus of Sheath

a. Small spores of 3—4 micra in groups.	*a.* Small spores of 3—4 micra in mosaic pattern, polygonal.
b. Chains of spores.	*b.* None
c. Septate mycelia of 2 micra.	*c.* None

5. Within the Hair

a. Mycelium straight or slightly curved.	*a.* Wavy branching mycelium of 2 micra.
b. Chains of spores of mycelia.	*b.* None
c. Small groups of spores.	*c.* None
d. Filamentous mycelium terminate in a fringe above the bulb.	*d.* Mycelium increased toward the root, terminates in a fringe of Adamson.

Negroni *and* Aberastury *studied a case of alopecia parvimaculata of trichophytic origin.*

They observed a case of Kerion Celsi caused by *Trichophyton ectothrix Microides* in a boy who on the height of the disease presented a dissemination of the parasite first by lymphatics, later by blood stream and as a consequence a lichenoid trichophytid in the cubital region and an alopecia parvimaculata on the scalp.

(2) *Allergy*

Arthur T. Henrici *carried out noteworthy investigations concerning experimental trichophytid in guinea pigs.*

The experiments reported have been carried out with a single strain of *trichophyton mentagrophytes* (*Tr. gypseum*) typical in all respects, isolated from a human infection of the nape which was probably contracted from a horse. Guinea pigs were inoculated by scarifying some of the fungus into the skin of the abdomen (previously depilated by barium sulphide) with sandpaper. Signs of infection were noted on the fourth or fifth day; the lesions extended a centimeter or two beyond the scarified area, and reached their maximum development about the tenth day; they were usually completely healed after four or five weeks.

To provoke an allergic reaction in the infected guinea pig, they have been reinoculated with live spores, or with cell sap expressed from the mycelium, or with crude polysaccharide extracted from the mycelium. Spore suspensions were obtained by washing the surface of large (25 mm) Sabouraud agar slant cultures with physiological salt solution. Cell sap was obtained by collecting the mycelium from 7-day-old cultures in Sabouraud's liquid medium, squeezing it as dry as possible, mincing it finely by repeated passage through a foodchopper, and expressing the sap by means of a hydraulic press. The liquid so obtained was passed through a Seitz filter. The remaining mycelium, now nearly dry, and weighing about 150g, was suspended in a liter of 1 % NaOH solution and heated just to boiling. Hydrochloric acid was added to the alkaline extract (separated from the mycelium) until a voluminous precipitate formed. After filtering, the liquid was added to 10 volumes of alcohol. The resulting precipitate was collected and disolved in 100 cc of water. Crystals of trichloracetic acid were added until no further cloudiness appeared. After removing this precipitate by filtering, the remaining liquid was neutralized and added to 10 volumes of alcohol. The resulting precipitate was collected and dried. The precipitate was called *crude polysaccharide.*

Twenty-two infected guinea pigs have been reinoculeda *intraperitoneallv* with live spores, in doses of either onehalf or one agar slant, at intervals from 10 to 52 days after the primary scarification. All of these exhibited some degree of reaction, but sensitivity is greatest at the time when the primary lesion has nearly, but not entirely healed, i.e. about 25 to 30 days. Such animals show 24 hours after the administration of the ,,shock dose'', a marked erythema observed on all exposed areas of the skin (the depilated area of the primary lesion, the paws, ears, scrotum, nose and lips). Desquamation is complete in 3 to 5 days. In from 2 to 5 days there occurs desquamation of the paws, the skin peeling off in large plaques. Simultaneous with the peeling of the paws, the ears become covered with fine scales, which may persist for a week.

Animals inoculated earlier or later than the period of maximum sensitivity have all reacted to a lesser degree. 14 noninfected animals inoculated intraperitoneally with live spores have failed to show skin reactions of any sort.

Four sensitized guinea pigs were given 5 cc of *steril cell sap* intraperitoneally. They reacted in a manner identical with those which had been reinoculated with live spores.

Two guinea pigs were given 50 mg of polysaccharide intraperitoneally. They showed general eythema followed by desquamation precisely as in those reinoculated with the live spores. Three additional animals received 10 mg of polysaccharide subcutaneously, and showed similar general reactions.

Two depilated noninfected controls were inoculated intraperitoneally with 5 cc of cell sap, and 2 others with 50 mg of polysaccharide, without any observable reaction.

Fungi in scales were looked for from the paws and ears and from the abdomen distant from the primary lesion without success in 6 cases. Sections of skin from paws and ears stained by GRAM-WEIGERT method have failed to reveal any fungi in 10 cases. Blood cultures have been made from 4 sensitized animals at intervals from 4 to 48 hours after intraperitoneal inoculation of live spores. They remained sterile.

These experiments strongly support the theory that trichophytid is an allergic response of the skin to substances in solution distributed to it by the blood.

E. WIESMANN reported on *trichophytids*. A patient suffering from sycosis barbae for five weeks after having had trichophytin injections every second day, developed an urticaria and a lichen trichophyticus. The writer thinks the urticaria, very rare as a complication in trichophytosis, may have been caused by infection through ascaris. After eliminating the ascarides the urticaria disappeared and could not be reproduced by new injections of trichophytin.

In a second case of trichophytosis the writer found a conglomeration of mycelia without any reaction surrounding in the intermediary sinus of a lymphatic node — except in a few of the slides. Along the periphery of the conglomeration, the mycelia showed signs of lysis without any apparent cellular reactions. The cultures from the lymphatic node remained negative probably because the fungus had been changed by the allergic process.

(*e*) *Microsporum*

Kerion celsi of the scalp in adult caused by *Microsporum*, is rare in all parts of the civilized world and especially so in the United States. J. A. BENSON reported the fourth case in the American literature of Kerion of the adult scalp (Negress aged 51) and the sixteenth of either the Kerionic or the superficial type of ringworm in which the diagnosis was proved by culture or microscopic examination. Microsporum infection of the adult scalp has occurred in Negroes in seven instances, being of the Kerionic type in four. General examination revealed no eruption of the glabrous skin. The lesion of the scalp consisted of a circumscribed tumescence 7 by 5 by 1.5 cm., located just to the left of the midline, overlying the medio-posterior portion of the parietal bone on the left. The surface of the tumor was irregular, and most of the scalp hair was missing. *Microsporon lanosum* was identified from the lesion by gross and microscopic examination.

Another case of *Microsporum* infection of the scalp in a white woman aged 30, was observed by PAUL E. BECHET. She had a patch of eruption on her scalp which had been present for approximately a month. In the left parietal region a circular patch about 3.5 cm in diameter was present. It was sharply defined and covered with scales and broken hairs. *Microsporum lanosum* was observed on scrapings from the lesion and on cultures. Trichophytin reaction was strongly positive.

G. M. LEWIS observed a similar infection in a Negress aged 14, having a profuse scaling over the scalp of from six to eight weeks duration. Cultural examination showed *Microsporon Audouini*. There was a negative reaction to trichophytin after twenty minutes, after 48 hours and after one week. The regularity of the menses was established one year ago.

G. BASSO and FLAVIO L. NINO observed a case of microsporosis of the scalp caused by *Microsporum felineum* in a 7 year old child in Mendoza.

P. A. MAPLESTONE and N. C. DEY described the fungous infection caused by a *hitherto unknown microsporon in India*. In India, microsporon infection of the scalp is uncommon among children residing in the plains. Practically all the cases seen are European and Anglo-Indian children coming from hill-station schools and as far as known no case has been reported in an Indian child. Out of 177 cases of ringworm of the scalp clinically and mycologically investigated in the Calcutta School of Tropical Medicine, sixty (51.3 %) were caused by Microsporon infection. The causative organism is usually the *Microsporon Audouini* in India. The basis of this study is made up from seven cases, girls and boys, from 6 to 9 years of age. The size of the patches on the scalp varied from a tiny spot infecting a few hairs, to patches 2 or 3 cm in diameter. Microscopically the hair showed small spores with typical mosaic distribution similar to that of *M. Audouini*. The culture, however, yielded a fungus the morphology of which is similar to that of *M. ferrugineum* Ota, 1921. The fungus grew slowly and showed two types of growth, a raised faviform type and a flat type. The color of the cultures showed various shades of brown whereas brown has only occasionally been recorded in *M. ferrugineum* Ota in which deep yellow is the most usual. The author thus considers this fungus as a possible variant of the *M. ferrugineum*, Ota.

ELEANOR SILVER DOWDING and HAROLD ORR gave a fine analysis of the *dermatophyte Microsporum lanosum* as found in the Canadian province *Alberta* As known, *M. lanosum* is capable of infecting the hair and the glabrous skin of animals and of human beings. In man it may produce ringworm of the scalp, ringworm of the body, and most rarely ringworm of the beard. However, in the survey of the dermatophytes of Alberta, they have repeatedly isolated *M. lanosum* from non-inflammatory type of ringworm due to *M. lanosum* is in fact clinically indistinguishable from that due to *M. Audouini*. In the present investigation, CONANT's rice medium was used. It is important to emphasize that the character of the mycelium of a dermatophyte varies with the composition of the medium and the age of the culture. This is illustrated by the following observations:

(1) Two cultures were used, both derived from the same patient, and of the same age, but one was grown upon rice and the other upon SABOURAUD's medium. The lengths of one hundred macroconidia from each culture were measured and frequency graphs were constructed. The mean length of spores formed on rice is 90 μ (with a standard deviation of 10 μ), and that of spores on SABOURAND's medium is 119 μ (with a standard deviation of 10 μ), a difference of 29 μ.

(2) Two cultures were used, both derived from the same patient, and grown on SABOURAUD's medium, but one had been growing for twenty days and the other for six weeks. The lenghts of one hundred spores from ecah cultures were measured and the mean length of spores of the old culture is 79 μ (with a standard deviation of 12 μ), while that of spores of the young culture is 95 μ (with a standard deviation of 10 μ), a difference of 16 μ.

Two strains of *M. lanosum* have been isolated in Canada, one with short spores and one with long spores. The two strains produce the same clinical type of ringworm. No correlation could be discovered between the strain of M. *lanosum* and the clinical type of ringworm which it caused.

The microconidia and the macroconidia, both of which are produced upon artificial media, possess different dispersal mechanisms. The microconidia are set free by the dissolution of the conidiophores and collect in slimy masses. The macroconidia are set free by the rupture of certain cells termed abscission cells, and they collect as a powdery deposit over the surface of the culture.

MASON emphasized that descriptions of spores should include their ,,dispersal mechanism'', i.e. whether they are ,,dry'' spores adapted for distribution by air or ,,slime'' spores adapted for distribution by water or another such agent.

The microconidia of *M. lanosum* are produced most abundantly in wet cultures. They collect in the moisture excreted by the aerial mycelium and a smear of this liquid on a glass slide contains large quantities of them. They are clearly ,,slime'' spores. The macroconidia, on the contrary, are set free by the rupture of a special cell, and since they collect as a powdery deposit on the surface of the culture, they are clearly ,,dry'' spores adapted for dispersal by air.

The authors observed abscission cells at the base of the macroconidia of *M. Fulvum* and *T. Gypseum.* It is probable that the macroconidia of dermatophytes are regularly ,,dry'' spores.

The powdery spore deposit of *M. Lanosum* becomes overgrown by a close-textured white down, termed ,,secondary mycelium'' which is composed of narrow, peculiarly thickened, almost sterile hyphae. The secondary mycelium when it is still young may be transformed to the original primary condition by transfer to fresh medium but when it is older it remains in its secondary condition even after transfer. The secondary mycelium usually originates from the base of the abscission cell after the spores are shed. The secondary mycelium may be produced experimentally before the spores are produced, by rounding the primary mycelium.

NEGRONI, PIERINI *and* CORDERO (1940) *reported on alopecia parvimaculata of microsporic origin.*

They observed two cases of ringworm of the scalp produced by Microsporum accompanied by ,,alopecia parvimaculata'' in children of 5 and 8 years of age, respectively. In the first case there appeared at the same time a lichenoid eruption (micropsorid) on the trunk, upperarms and the thighs. In the second case they have noticed an enlargement of the regional lymph-glands without inflammation. They could not find any sign of suppurative process in the present condition or in the anamnesis. The patches of ,,alopecia parvimaculata'' were irregular, small, white, and smooth, with ill-defined edges and without inflammation. They believe as WERTHER (1931) that in these cases the ,,alopecia parvimaculata'' represents a microsporid of the scalp.

(*f*) *Favus*

REQUE, BAREFOOT *and* CONANT *reported a case of tinea favosa of the scalp from North Carolina.*

Although in recent years several cases of tinea favosa of the scalp have been reported in native-born Americans the number is relatively small when compared with the incidence of the disease in foreign-born Americans. Most of the cases reported among native-born Americans are in the offspring of foreign-born parents who have, or have had the disease.

The case to be reported is, to our knowledge, the first published report of tinea favosa in a native-born North Carolinian. The main point of interest is the problem presented in the diagnosis of the disease. Altough favus is rare, it probably often goes unrecognized, owing to the possibility of confusion with other diseases of the scalp, notably seborrheic dermatitis, psoriasis, and tinea amiantacea.

There has been no satisfactory explanation for the cases reported from isolated areas in Kentucky, Arkansas, Tennessee, and in North Carolina. Apparently in most of the reports the patients lived in poverty stricken districts. The patient herein described was supported by local welfare agencies.

The possible difficulties encountered in the diagnosis of tinea favosa, or favus of the scalp, are considered, and a case of the disease in a native-born North Carolinian is reported. There was no known contact with foreign-born persons, but a brother of the patient has a similar condition of the scalp. The duration of the disease is twelve years. Methods of diagnosis and treatment are discussed.

H. W. SIEMENS and A. J. M. KRENS reported on *favus* in the schools in Holland. Among the fungus diseases beside Tinea trichophytina and microsporina, favus still holds the first place. Holland's eleven provinces comprise 1070 communities from which 582 or 54 % showed the presence of favus. In the campaign against this stubborn fungous infection, the author asks for the proper training of the physicians, the enlightment of the public and above all for the enlightment of the civil administrative and school authorities. He urgently advocates the appointment of school physicians in the entire state (at present there are only 25 % school physicians), and an ordinance for all communities by virtue of which the school attendance of children infected with favus is only permitted with tightly closed head-dressings if and when the child is under regular and permanent medical care. This type of sufficient control measures is at present only for 4 % of the 1070 communities in Holland available. The authors further asks for a police ordinance for adults sick with favus forbidding them any kind of occupation in surroundings where they may become sources of infection.

C. MARCHI reported on the extremely rare case of a *septicemia due to Achorion schoenleini*. The patient was a 10 year old boy suffering from favus of the scalp for the last three years. The scalp was entirely covered like a tight cap with scutula. Up to his admission the course of this subborn mycotic disease was ,,normal'', without any particular signs or symptons. One month before admission, however, he fell gravely ill, characterized by cephalalgia, arthralgia, anorexia, severe chills. This symptomatology was accompanied by a progressive deterioration of the general health and nutrition, and a serious bronchitis with disturbing cough developed. The temperature ran high, up to 40.3° C with remissions. He developed a secondary anemia (red cells: 3 million, HB. 56 %, white cells: 18,000), subicterus, tumor of the spleen, hepatomegalia, diarrhoea, meteorism, albuminuria and epistaxis.

In the course, above all, typhoid fever, miliary tuberculosis, malaria, and Malta fever (febris melitensis) were by thorough examination excluded. Common pyogenic sepsis was also disproved. The blood culture yielded, however, the *Achorion schoenleini* in pure culture on SABOURAUD's glucose and maltose media. Sporo-agglutination and the complement deviation test with the same fungus were positive. Trichophytin test was negative.

Later on the patient developed a pleuritis dextra in the exudate of which the *Achorion schoenleini* again was present in pure culture. He developed a pleuritis on the left side whose exudate proved to be culturally also positive for the fungus. All this condition was joined by a heart dilation due to an acute myocarditis.

The patient shortly after his admission showed, moreover, in different form *favids* of the skin. The seventh day of his hospitalization he developed a generalized, vesicular eruption all over the body which disappeared spontaneously in a few hours afterwards. The eleventh day he suddenly revealed a morbiltiform rash in the abdomial region which again disappeared spontaneously after a short duration. On the 25th day he showed on the whole trunk and abdomen typical roseola vanishing quickly.

Due to the vaccine therapy (tineatoxin), the life of the patient was saved.

It is not uninteresting to recall in this connection, the few, rare evidences of a generalized favus-infection recorded in medical history. NOBE observed a case of generalized favus infection with arthropathia of the right knee. *Achorion schoenleini* was demonstrated in his feces. KÜNDRAT and KAPOSI made a postmortem of a 40 year old man who suffered for 30 years from favus and who died from generalized favus in connection with a phlegmone of the popliteal region, ulceration of the gastric and intestinal mucosa covered with favus colonies.

There are a few noteworthy case reports on favus.

P. PHOTINOS and CH. VOSSYNIOTIS observed and studied the first cases in Greece in which favus was coexisting with Tinea trichophytina of the scalp. The patients were two brothers, 5 and 6 years of age, respectively. The clinical picture was characteristic with scutulum fromation and scarring. The foci yielded culturally *Achorion schoenleini* and *Trichophyton violaceum*, the latter being the most frequent agent of trichophytosis in Greece.

In the case of H. GOUGEROT, P. BLUM and DUCHE, *Achorion schoenleini* was associated with *trichophyton crateriforme*. The boy, 11 years of age, borne in Algier revealed erythematosquamous, crusted lesions of the scalp, partly impetiginized.

A. MARTINS DE CASTRO observed as infecting agent the *Achorion Gypseum* in a boy 8 years of age, who showed a quickly spreading inflammatory process of the upper and partly the lower lid of the right eye. The lesions were crusted, harboring in between little pustules. It is interesting that the diagnosis first was made as trichophytosis until the culture revealed the true nature of the infecting agent. *A. Gypseum* is more rarely found in Brazil than the *A. schoenleini*.

LUIS E. PIERINI treated 600 cases of favus with Thallium Acetate (Merck). The dose administered was 8 mgm per Kgm of body weight. The hair fall started about 10 days after the dose given, reaching the height about the

14th day. The treatment consisted afterwards in application of salves, in washings with hot water and soap and in between with rubs with 1% Tct. of Iodine. Untoward effects were noted as muscle and joint pains, slight fever, conjunctivitis, anorexia, anemia, rarely albuminuria. All these symptoms were not long standing. The author recommends this procedure not only for specialist but also for the general practitioner as the method of choice.

Against this attitude we have to raise again our warning voice with regard to the extreme toxicity of the drug and in consideration of the numerous fatal cases, in addition to those who paid for the ,,cure'' with this drug with permanent idiocy. The drug is certainly not for the general practice.

Erich Hoffmann called attention to the centenary of the discovery of the first vegetable organism of a human contagious disease, the *Achorion schoenleini* (1839). He gave a historical review of the powerful personality and work of Schoenlein, this great clinician, who broke the rule of the ,,natural philosophers'' (Naturphilosophie) representing the lowest ebb in the history of German medicine. Schoenlein introduced laboratory methods, experiments, the use of the microscope in dealing with the sick and he became the head of the school of ,,Natural history'', introducing the modern era of clinical medicine based on observation, and experiments in Germany.

The center piece, of course, is taken in Hoffmann's dissertation by the description of the discovery of the favus fungus by Schoenlein. It is amazing to observe, the certainly undeserved glorification of Schoenlein beyond any measure in connection with the discovery of the favus fungus, and to put Schoenlein in the same rank with Agostino Bassi whose discovery, description, experimental and microscopic investigations on the muscardine of the silkworm caused by the *Botrytis bassiana* are and remain a masterpiece of microbiological research.

The discovery of the favus fungus was many times since its beginning matter of controversy. Beyond doubt Schoenlein saw the agent of favus, but discovery is something different. Schoenlein never did it. To let the unbiased reader judge this controversial matter, I give here first a reproduction of the original publication of Schoenlein followed by literal translation. Thereafter I let follow the communications of David Gruby sent to the French Academy dealing with the same matter.

The translation of Schoenlein's original communication:

"On the pathogenesis of the impetigines".[1]
by
Prof. Schoenlein in Zurich
(Excerpt from a communication by letter to the editor)[2]
(With Table III. Fig. 5).

You know beyond doubt the beautiful discovery of Bassi on the true nature of the muscardine. This fact appears to me of the highest interest for the pathogenesis although as far as I know no physician appraised it with his attention. I had, therefore, numerous samples of silkworms, suffering from muscardine, sent to me from Milan and my experiments carried out with them substantiated not only Bassi's and Audouin's communications, but they yielded some more not quite insignificant results. Thereby I was made thinking again of my opinion of the vegetable nature of some Impetigines, an opinion, which received a forceful support previously through the beautiful work of Unger on plant exanthema. Since I fortunately just had a few cases of *Porrigo lupinosa* W(illan) [3] in the hospital, I made closer investigations, and the first examination left no doubt about the fungus nature of the so-called pustules [1]). Enclosed there is a microscopic picture of a portion of a pustule. The same time I send you a few Porrigo-pustules removed with the greatest ease from the upper layer of the cutis in the living. I am busy with further investigations on this object, the result of which I soon intend to publish".

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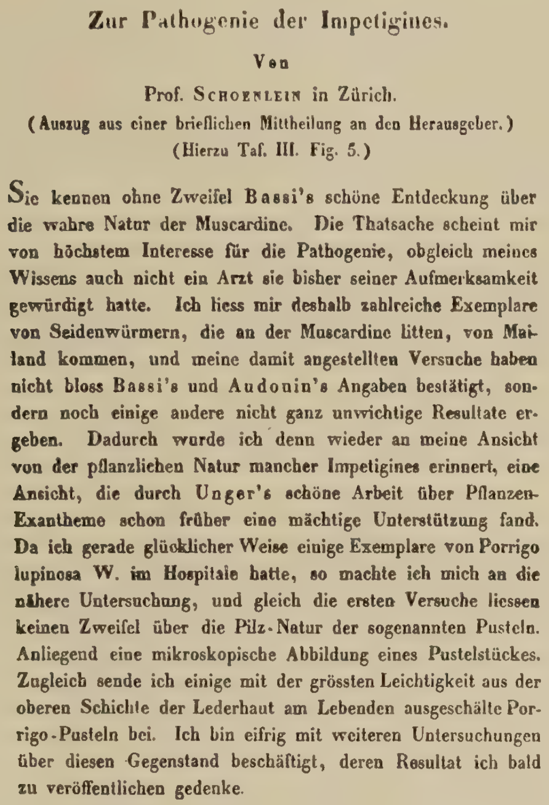
[1] The systematic dermatology put at Schoenlein's time favus in the group of impetigo.
[2] Muller's Arch. Anat. Physiol. Wissensch. Med. 1839, p. 82.
[3] The famous British dermatologist, the founder of modern dermatology.



Zur Pathogenie der Impetigines.

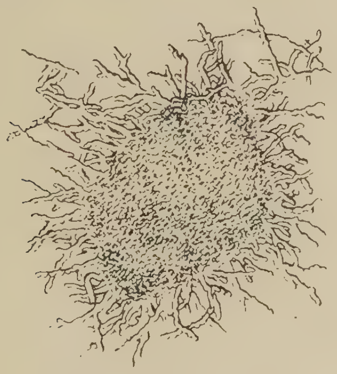
Von

Prof. Schoenlein in Zürich.

(Auszug aus einer brieflichen Mittheilung an den Herausgeber.)

(Hierzu Taf. III. Fig. 5.)

Sie kennen ohne Zweifel Bassi's schöne Entdeckung über die wahre Natur der Muscardine. Die Thatsache scheint mir von höchstem Interesse für die Pathogenie, obgleich meines Wissens auch nicht ein Arzt sie bisher seiner Aufmerksamkeit gewürdigt hatte. Ich liess mir deshalb zahlreiche Exemplare von Seidenwürmern, die an der Muscardine litten, von Mailand kommen, und meine damit angestellten Versuche haben nicht bloss Bassi's und Audouin's Angaben bestätigt, sondern noch einige andere nicht ganz unwichtige Resultate ergeben. Dadurch wurde ich denn wieder an meine Ansicht von der pflanzlichen Natur mancher Impetigines erinnert, eine Ansicht, die durch Unger's schöne Arbeit über Pflanzen-Exantheme schon früher eine mächtige Unterstützung fand. Da ich gerade glücklicher Weise einige Exemplare von Porrigo lupinosa W. im Hospitale hatte, so machte ich mich an die nähere Untersuchung, und gleich die ersten Versuche liessen keinen Zweifel über die Pilz-Natur der sogenannten Pusteln. Anliegend eine mikroskopische Abbildung eines Pustelstückes. Zugleich sende ich einige mit der grössten Leichtigkeit aus der oberen Schichte der Lederhaut am Lebenden ausgeschälte Porrigo-Pusteln bei. Ich bin eifrig mit weiteren Untersuchungen über diesen Gegenstand beschäftigt, deren Resultat ich bald zu veröffentlichen gedenke.



This is the only original communication of Schoenlein on the "fungus" of the favus. Schoenlein saw the vegetable material of the favus "pustule", he even assumed its vegetable nature, but it was not a discovery at all. Even the illustration to this "discovery" consisting in two (2) sentences, is neither help nor substantiation of the discovery because being a very schematic drawing does not give any essentials from the fungus. SABOURAUD expressed his opinion also about the mediocrity of this illustration.¹)

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[1]) "pustule" . . . favus scutulum.

SCHOENLEIN did not describe the pathogenesis of the scutulum (then pustule of the *porrigo lupinosa* Willan), he did not describe the morphology of the fungus, he did not describe the fungus in the hair, he did not describe the subsequent changes in the skin. I wonder, where is here his discovery.

ROBERT REMAK had a peculiar connection with this question. He already differentiated in 1837 the filamentous structure of pustule of porrigo lupinosa (favus scutulum) from the other "Impetigines". Peculiarly he did not publish these findings. They were only mentioned in the dissertation of his friend XAVER HUBE saying that the "pustule" consists of "fibrae ramificatae" (ramified fibers) and of smallest "corpuscula" (corpuscles, (the spores, B.)) in the "crustis flavis tineae favosae". REMAK as clinical assistent of SCHOENLEIN carried on the further study on favus announced in 1839 by SCHOENLEIN. Eventually REMAK ²) in 1845 . . . six years after SCHOENLEIN's announcement and four years after DAVID GRUBY's classical and unsurpassed description of the favus scutulum and favus fungus in 1841 . . . published his investigations on spore formation of the fungus with successful culture of it on a slice of apple and with a positive inoculation of the fungus on his own arm.

HOFFMANN with his unwarranted insinuation and disparaging remarks of GRUBY as the real discoverer of the fungus of the favus, could not help his cause and could not make out of a 2-sentences vague communication a great discovery. ³)

To give the reader opportunity to compare Schoenlein's communication with DAVID GRUBY's description on the topic let us follow a literal translation of GRUBY's communication to the French Academy.

On a vegetation causing the true tinea⁴) ⁵)

The most distinguished medical men are of a contrary opinion about the nature of the tinea and its site. We quote but the commonest opinions; WILLAN and BATEMAN, and RAYER, adopt a pustule as a primary lesion, while BAUDELOCQUE, MAHON and GILBERT deny the existence of these pustules as elements of the tinea. The same dissention prevails as to its contagiousness. ALBERT denies the contagiousness, contrary to the majority of medical men admitting it. The same way, MAHON and many others consider the follicles of the skin as the site of the disease, while BAUDELOCQUE puts it into the bulbs. The diagnostic is very difficult in many cases in want of essential characteristics, and it is not seldom to see the true tinea confounded with the pseudo-tinea and many others of its kind. The diagnostic was based on the following more or less constant characteristics:

(1) On the pustules placed under the epidermis around the hair; we have seen, however, the disagreement of the observers on this point, and we have, indeed, seen several cases of true tinea without pustules;

(2) On the depression caused by the round yellow crusts; one can not use, however, this characteristic provided the crusts are well developed and still unimpaired, what is very rare;

(3) On the contagiousness; but, besides that it is not yet well established, it would be very inconvenient to inoculate such a serious disease for diagnostic purpose;

(4) On specific odor, which can not be detected neither at the beginning of this disease, nor in those cases with small extension.

All these characteristics being insufficient in order to establish the true tinea, I shall expose in this paper:

(1) A new characteristics of the true tinea, fairly constant and clear cut, to become diagnostic characteristics of this disease;

(2) The physiology of the pathologic product;

(3) The special site and the relationship existing between this morbid product and the surrounding tissues.

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¹) SABOURAUD, R.: Les Teignes, Masson & Co., Paris, 1910, pp. 7.

²) REMAK, Diagnostische and pathogenetische Untersuchungen, Berlin, 1845, VII Muscardine and Favus (Porrigo lupinosa), p. 193—215.

³) It is characteristic, that HOFFMANN purposefully omits in his quotation of literature the work of my great teacher, J. H. RILLE on DAVID GRUBY. (Dermat. Wchschr. 82: 512—516, 1926).

⁴) Author's extract in Compt. rendu Acad. Sc. 13: 72—75, 1841, presented in the meeting of the Academy, Mon., July 12th, 1841.

⁵) At the time of GRUBY "true tinea" meant favus.

(I) To recognize the true tinea one has only to put it under the microscope; for this purpose one can use a small portion of the crust mixed with a drop of pure water; one puts it between two slides, and one examines it under an enlargement of 300 diameters. One will see there a great quantity of round or oblong corpuscles, the longitudinal diameter of which is from 1/300 to 1/100 of a millimeter, and the transverse diameter from 1/300 to 1/150 of a millimeter; they are transparent, with clear cut edges, with smooth surface, colorless, slightly yellowish and homogenous. In addition to this, one observes small septate filaments with a diameter of from 1/1000 to 1/250 of a millimeter, transparent and colorless; in general, the form of these filaments is cylindric or branched according to the portion of the crust to which they belong.

The cylindric filaments are composed of oblong or round corpuscles, often showing the aspect of beads¹); the branched filaments, on the contrary, reveal transverse septa in intervals, representing oblong cells in which one finds small, round, transparent corpuscles of from 1/10.000 to 1/1000 mm in diameter. At times one finds granules adhering to the filaments, similar to the spores of *Torula olivacea* and *Torula sacheri* (sic) depicted in the volume titled: Icones Fungorum of Corda (Pragae, 1841, vol. IV). The form of these filaments establishes their character as vegetables beyond any doubt; they belong to the group of Mycoderma according to BRONGNIART.

Since we have not yet found any minute particle of the true tinea not being heavily loaded with this mycoderma, they constitute a true, essential characteristics of this disease.

(II) The crust of the tinea offers some very interesting peculiarities to pass over silently. One had to choose an isolated, unimpaired crust of only few weeks old, the external surface of which is not crusted; therefore, one will take from a spot where the hair do not interfere with its easy removal. The crust reveals then the aspect of a flat capsule, like the shell of nux vomica, to wit, the form of a disc one of the surfaces of which is concave, the other one convex. The circular edge is divided by a shallow furrow in two equal parts, the superior of which is exposed to the air, and the other one turned to the skin. These two discs are of yellow color at their external surface and of grayish-white at their interior. The concave surface is the aerial portion, the convex one is the dermal part.²)

The whole crust is wrapped in epidermal cells being much greater in number at the aerial than at the dermal surface.

There is also a second envelope wrapping up the whole crust, being composed of particles of different size which form an amorph substance placed between the epidermal cells and the parasitic vegetation itself. In the interior one then finds the vegetable parasite, the roots of which are placed immediately in the amorph substance just mentioned; the ramification, on the contrary, continues toward the center of the crust. A vertical section of the crust reveals a central porous, grayish, friable tissue composed of granules and branches of the mycoderma; granules there outnumber the branches very much. In the peripheral portion, on the contrary, one sees the conpact tissue of the amorph substance, where the roots of the mycoderma are placed.

The granules appear to be the products of the vegetable and they probably serve to its propagation.³)

Summing up, each isolated crust of the tinea consists of two envelopes and of a collection of the mycoderma being there enclosed like the fruits in their pericarpes.

The contagiousness of this disease becomes more probable on account of its vegetable nature, and I shall have the honor to submit to the Academy the results of inoculation of this vegetable in humans and in different classes of animals.

(III) The epidermal tissue is the special site of the tinea; the epidermal cells which due to their structure approach the structure of vegetable tissue, seem to be able to give birth to a similarly vegetable tissue; one sees thus the mycoderms of the tinea to vegetate between the cells of the epidermis, in only compressing the tissues of the skin without destroying it. At times one finds the cutaneous tissue inflamed under the crust with few inflammatory cells.

One almost always can remove the crust without hurting the skin.

In the most frequent case, after the removal of the crust, a serous fluid exudes on the surface of the wound to cover it; one sees thus the healing accomplished scarring, which seems to prove that the skin was not destroyed neither by the suppuration nor by the ulceration.

The connection between the hair bulbs and the parasites is not so intimate as one had assumed; because it often happens that the mycoderma is well developed without the hair follicles being substantially altered. At times, however, the filaments of the mycoderma invade the hair follicles and envelope the bulbs which causes the conical shape of the dermal portion of the crust.

The hair then is softened to such an extent that, put between two slides, a slight compression suffices to make it flat and to split it in the direction of its fibers which easily permits

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¹) Description of the chlamydospores. (Annotation of B.)

²) Description of the favus scautulum. (Annotation of B.)

³) Excellent observation of the spores of the favus vegetation present in the scutulum and of its nature as organs of reproduction. (Annotation of B.).

the study of the filaments. The follicles of the skin are secondarily attacked by the same alteration, like the other tissues of the skin.

Concerning the therapy of this disease, these new facts may engage the practitioner to make new effort in this direction.

On the mycoderma causing Tinea Favosa [1]. [2]

Mr. Gruby, on occasion of the claim of priority raised recently in favor of Mr. Schoenlein concerning the discovery of the vegetable nature of certain exanthems, writes that at the time when he made his first investigations on the subject, and even up to date, the investigations of Schoenlein were completely unknown to him.

"The results I reached, Gruby said, seem, moreover, to differ in every essential points from those announced by Schoenlein in Mueller's Archives. Schoenlein speaks of a pustule on which he saw a vegetation:, I, on the contrary, never found the mycoderma on a pustule, and even I deny the existence of pustules in the tinea favosa, in which (pustules) I do not see anything but the collection of millions of mycoderma[3]); therefore, I attached a great diagnostic value to the existence of the mycoderma, because I never encountered a bit of tinea not presenting it. I showed that the mycoderma is placed between the epidermal cells and that the suppurative stage or the ulceration of the skin, admitted nowadays by all pathologists, have in fact nothing to do with; finally that the follicles of the skin are secondarily affected by this disease. I described the mycoderma in all its details and even I let know its mode of reproduction".

"The famous pathologist of Berlin [4]) has found, on his side, a cryptogamic vegetation on the *Porrigo lupinosa*; but he did not describe it, he did not discuss the details of its site; he did not tell, what is of the greatest importance, that the whole disease of the tinea is nothing but a vegetation; he did not tell that the constant and essential character for the diagnosis is the vegetation".

"What I recall suffices to show that there is, so to speak, no relationship between my observations and those of Schoenlein. Today I am going to complete my studies on the tinea, in exposing: (1) the different degrees of development of the mycoderma's capsule; (2) the results produced by certain chemical reactions; (3) the results of some experiments of inoculation".

"The peripheral disc of the capsule which is not perforated at the beginning opens at the center through a little hole the border of which is raised by the continuous development of the mycoderma; the hole becomes larger little by little, and one can see at the center a white excavation while the borders of the capsules are of yellow color; in the same time when the hole becomes larger, the mycoderma, placed in the capsule, arises and develops as a fungus until the borders disappear completely; the stems of the mycoderma lengthen, and the spores multiply vigorously, more in the center than at the periphery; hence a form results which is the reserve of that observed before the capsule was open: then there was a depression in the center; in the last stage of development, on the contrary, the center is more protuberant than the borders. One sees this complete development in young well nourished individuals and when the capsule of the mycoderma was not crusted".

After having described the results obtained in treating the mycoderma of the tinea with different reagents, the autor goes over to the inoculation experiments made, experiments, though very numerous, did not give the results which he believed to be able to obtain.

"I carried out, he says, inoculations on thirty phaneroganic plants; I did not succeed but once. On 24 silkworms, I did not obtain any results. On six reptiles, I did not get any results, either. On four birds and on eight mammals, no results. In my first experiment on humans I inoculated Prof. Rinneker, of Wurzburg, on the arm; this inoculation produced a slow inflammation and a slight suppuration. I inoculated myself four times with the same results. In total, in 77 inoculations I did not obtain a result but on one plant; this unique fact, however, seems to me inquisitive in giving an example of a human disease communicable to a vegetable".[5])

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[1]) Compt. rendu Acad. Sc. 13: 309—311, 1841, presented in the meeting Monday, August 2, 1841.

[2]) This is the second paper of Gruby on favus, sent in the form of a letter to the Academy. Gruby gives here a more detailed account of his first and epoch making discovery, refuting Schoenlein's claim of priority. (Annotation of B.).

[3]) "Mycoderma" equals our present general term: fungus. (Annotation of B.).

[4]) Schoenlein (Annotation of B.).

[5]) A most remarkable observation and discovery of the fact that (human) pathogenic fungi are able to vegetate on living plants. (Annotation of B.).

(g) *Epidermophyton and „Epidermophytosis"*

E. M. BURLINGAME and G. F. REDDISH (1939) surveyed the laboratory methods for testing fungicides used in the treatment of epidermophytosis. They form probably the first laboratory method of merit proposed by SCHAMBERG and KOLMER in 1922. Since the laboratory methods of tests available did not seem practical enough to indicate whether or not the fungicides tested would be effective under clinical conditions of use, they found it necessary to develop a new severe test which more nearly approaches clinical conditions met with in practice. It was found that those fungicides which pass this test within the minimum time period of the test are effective in the treatment of epidermophytosis and that those which do not pass the test in the maximum time period are not clinically effetive. In this study five such tests organisms were employed: *Epidermophyton interdigitale, Trichophyton purpureum, Trichophyton gypseum, Epidermophyton inguinale,* and *Trichophyton rosaceum.*

The fungicidal test was carried out as follows: using the test organisms. listed above, each organism is streaked over the entire surface of SABOURAUD's agar (20 cc) in a 9 cm. PETRI dish, using a dry sterile cotton swab inoculated with cultures of these organisms which had been grown on SABOURAUD's agar for five days at room temperature. These plates are incubated at room temperature for five days. The agar cultures are then cut into 1 cm squares, or into disks by means of a cork borer, 1 cm in diameter. The fungicide to be tested is poured over the surface of the cultures so as to entirely flood the plates, which requires 15 cc or more for each plate. After five, fifteen, and thirty minutes, one of the squares or disks of culture and agar from each plate is removed and placed into 10 cc of sterile broth. The excess fungicide is washed out of the matted culture by shaking the broth tube lightly for five minutes. At the end of this time the block of the culture is removed from the broth and spread over with the culture side down over the surface of a sterile slant of SABOURAUD's agar. These slants are then incubated at room temperature for three weeks and observed for growth. An effective fungicide should kill these tests organisms within five minutes.

It was found that those preparations (none is mentioned, B.) which kill these test organisms under the conditions of this test within five minutes are clinically effective in the treatment of this infection. Clinical tests on 30 patients suffering from „athlete's foot" showed that such fungicides are effective in clinical practice. Clinical tests on 30 other patients also showed that a preparation which did not pass this test — — — that is, did not kill these test organisms — in thirty minutes, was ineffective in the treament of epidermophytosis.

Ointments, salves, powders, etc., which are used in treating epidermophytosis may be tested for fungistatic activity as follows: Add 2 c.c. of sterile normal horse serum to 18c.c. of SABOURAUD's agar, which has been melted and cooled to 45° C., and pour into 9 cm. PETRI dishes. After the agar has hardened, streak each plate with one of the test fungi, using a dry sterile cotton swab. The preparation being tested is then added to the surface of the agar. Ointments and salves are melted and streaked on the surface of the inoculated agar by means of a glass rod, and powders are applied to the surface with a small spatula. These are applied to but a small portion of the plate, the ointment along a streak about 1.0 cm. wide and extending across the middle of the plate, and the powders applied to an area of about 1 cm. square. The plates are incubated at room temperature for five days. If the preparation tested is fungistatic, there will be a clear zone in the medium around the area where it was applied. The extent of this clear zone shows the amount of penetration and the effectiveness of the fungistatic ingredient.

P. K. Fraser (1938) reviewed the question of the diagnosis of superficial ringworm infections. In the Medical Service of the Royal Navy nomenclature of these conditions is still notoriously vague and haphazard. In statistical returns they are variously designated as mycotic infections, trichophytosis, pompholyx, and tinea, with the result that accurate statistical deductions are impossible. He considers the clinical diagnosis of pedal infections, nail infections, ,,distal eruptions'', the ,,ides'', and their microscopic diagnosis. The paper reflects the deep confusion prevailing in the whole question. It certainly is not the fault of the author who gives no original research. Typical superficial tinea trichophytina of the glabrous skin is confounded in the same breath with typical pompholyx of the sole and the matrix of the nails, the lichenoid id (trichophytid) of the upper extremity with the ,,dyshidrotic id'' of the fingers. There are, however, a few original observations worth mentioning. He emphasizes the primary lesion of pompholyx of the feet, between the toes, being a vesicular eruption in line with the correct observation of Witfield (1934) in whose opinion all cases of ,,pedal infections'' (pompholyx) commence as a vesicular or bullous eruption and contrary to the statement of Gray (1934) following the opinion of C. J. White (1927) in postulating a primary intertriginous type. The primary lesion of pompholyx of the feet wherever the localization may be, on the toes, between them, or in the instep and however quick and early they might be destroyed, is always the deep seated vesicle.

The second point where the author advanced his own opinion is in the question concerning the ,,mosaic fungus''. He refers to the observation of Dowding and Orr (1936) who considered this structure as a disorganized fungus with the normal fungus forming a part of a mosaic. He could substantiate this finding observing one case where the mycelium ended in a mosaic.

The reviewer spent for a long time special attention to this question which was more than duly discussed particulary in the American literature (Greenwood, 1935; Davison and Gregory, 1935; Swartz and Conant, 1936). The mosaic fungus is not an artefact, it is not due to some interaction between the epidermal cells and the potassium hydroxide used in mounting. One could say it is the shadow of the fungus in which the protoplasm is entirely dried up leaving only the walls and the septa of the mycelium. One could compare it with the shed skin of a snake.

H. W. Haggard, M. T. Strauss, end L. A. Greenberg (1939) reported on fungus infections of the hands and feet treated by iontophoresis of copper. Copper was selected as the metal for iontophoretic introduction into the skin because of its well known fungicidal properties and its low toxicity .The supposition that copper could be introduced through the skin was confirmed by experiments on rats. In treating human beings the feet were immersed in rectangular enamel pans about five inches deep and the hands in bain-Marie basins. Copper electrod were used. The duration of treatment was twenty minutes. When the infection was on the feet, the hands were immersed in saline solution and the feet in 0.2 per cent copper sulfate solution; for infection of the hands the relation was reversed, and if both hands and feet were infected the solutions in the baths were changed at the end of 20 minutes and treatment was continued for an additional 20 minutes. The positive pole of the source of current was attached to the electrode in the copper solution and the negative to that in the saline solution. From four to six milliamperes were employed if only one hand or foot was immersed and from eight to ten milliamperes if both were immersed. When possible, each patient was treated two or three times a week. During the entire period no other treatment was used. The preparation of the hands and feet for immersion was found to be particularly important. The infected area was often covered with a greasy exudate which was resistent to the passage of current precisely in the regions where penetration was desired. A preliminary soaking of the feet or hands for a few minutes in a dilute solution of sodium hypochlorite has been found highly efficatious in preparing the skin for treatment. Treatment was given to 37 patients. The diagnosis of dermatophytosis were made clinically. Twenty of the patients had shown clinical signs of infection for more than a year and one for 25 years. The majority of the infections were severe and of long standing.

The results of treatment were most striking in those cases in which the infection was particularly active and showed bullae and vesicles. In most instances subjective symptoms disappeared within 24 hours after the first treatment, and vesicles flatened out without rupturing. When the entire extremity showed dry, normal appearing skin, the treatment discontinued. Twenty-six, or 70 per cent, of the 37 patients obtained a clinical cure; the remaining eleven were still under treatment. The number of treatments necessary to attain a cure varied from four given over a period of two weeks to 18 given over a period of eight weeks. The average number of treatments was slightly more than six. Twenty-three of the patients could be followed up for periods varying from three to six months. Of these, five suffered recurrences of some degree. Two reverted to a condition as severe as before treatment was started. Three others had mild recurrences. One patient with the history of having had vesicles on his feet for 25 years, showed rapid improvement but relapse occurred after an almost complete clinical cure. One patient, despite twenty treatments over a period of two months, has shown no improvement. In another one a clinical cure of the lesions of the feet was attained but had persistent vesicles on his hands. One revealed, in spite of fifteen treatments, considerable maceration in the fourth interspace of each foot. Five patients presented, in addition to typical dermatophytosis, eczematoid lesions of the arms and legs. In three of these the eczematoid lesions disappeared while the feet were being treated. In the two others the lesions improved.

E. F. Traub and J. A. Tolmach (1939) reported on their attempt of treatment of dermatophytosis with trichophytin, convalescent or immune serum, and vaccines. They studied a large series of cases of dermatophytosis, dermatophytosis with ,,ids'', and tinea capitis. Sixty-five of these were here reported. They used three different vaccines, all of which have been described in the literature. The results were far from gratifying. Over sixty per cent of the cases were entirely uninfluenced by the treatment. About nine per cent were apparently cured. These experiments convinced the authors that vaccine treatment of dermatophytosis is not yet practical and that further experiments are necessary.

The authors emphasize — and we thoroughly agree with their views — that only those experiments can be accepted where the authors actually proved that they are dealing with cases of dermatophytosis. ,,Mycotic-like'' eruptions with a positive trichophytin test, cases of probable ,,ids'', and eczematous hand and foot eruptions clinically called dermatophytosis and dermatophytid (in the large majority of which it may be assumed that both the omnipresent Monilia and the almost omnipresent epidermophyton could have been found by careful mycologic investigation — but were not — are not the carefully selected type of cases on which we believe such a study should be based.

They do not feel that vaccine, trichophytin, or convalescent serum treatment of dermatophytosis should be discarded. They rather feel that the biologic methods of treatment still belong in the laboratory and should not yet be advocated for general use.

Alderson (1940) advised his patients with mycotic infections of the hands and feet to apply ordinary vinegar twice daily as a cleanser. This has proved to be a useful auxiliary to other treatment. It also has served as a valuable prophylactic measure in the ,,after-treatment'' of such conditions.

The author was informed by J. C. Belisario Sydney, Australia of his using successfully citric acid in the treatment of dermatophytosis. Belisario's patients were advised to rub the raw surface of a lemon cut in half into the affected areas. The author, while at sea, had an acute vesicular attack on one sole and one palm. For less than a week he rubbed in lemon several times daily, allowing it to dry. Aside from a temporary smarting and some ,,stickiness'' for a while, the treatment was not disagreeable. The lesions disappeared in a week, the process ending in peeling. A subsequent recurrence during hot weather in the mountains of California was promptly relieved with lemon juice, together with a salicylic acid — boric acid powder and finally a non-medicated neutral oxycholesterol-petrolatum ointment base. He used this treatment successfully in many cases the most striking of which was that of a student who had had the eruption on his palms more or less constantly for two years.

P. A. Neal and C. W. Emmons (1939) from the United States Public Health Service presented a comprehensive study on dermatitis and coexisting fungous infections among plate printers. In October, 1936 the Division of Industrial Hygiene of the National Institute of Health began a study of the above condition.

For a number of years plate printers employed in this establishment had suffered from recurrent lesions of the skin of the hands and forearms. In certain cases these lesions had rendered the employees totally disabled for their usual occupation for varying lenghts of time and also disabled for other work. These cases have been very resistant to treatment, even after removal from their occupational exposure, and several employees have had relapses immediately upon returning to their usual occupation. The majority of these cases had been diagnosed as dermatitis venenata. The exact substance responsible for the skin lesion was clinically undetermined. Many of these cases had also a certain irritant fungus infection of the feet. Therefore, the purpose of this study was to determine, (1) the potential skin hazards associated with this industry, (2) the incidence and type of the dermatitis among the employees of this establishment, (3) the exact etiological factor in each case of dermatitis, and (4) to develop methods of control.

In order to accomplish this purpose the following procedure was followed: An occupational analysis was made which was designed to present information on three factors: (1) Occupational data pertaining to the number of employees by occupation and their activities, (2) the materials used, and (3) the sanitary facilities.

After completion of the occupational analysis a medical examination of all plate printers was made in order to determine the type and incidence of the dermatitis. This examination included an occupational history, past and present medical histories, a thorough examination of the skin, and a mycological examination of material from the hands and feet. Following this part of the medical examination, patch tests with all the inks then in use at this printing plant were made on all employees who had a dermatitis of the hands at the time of the examination or who had a history of ever having had a dermatitis of the hands, as well as patch tests on an adequate number of controls. This examination was made in order to assist in the determination of the exact etiological factor in individual cases of dermatitis.

The mycological study was made in order to determine whether pathogenic fungi were either primary or contributing etiologic agents in the dermatitis.

After an examination of the skin of the entire body was completed special attention was given to an examination of the hands and feet for clinical evidence of fungous infection. A notation of clinical dermatophytosis of the feet was made when one or more of the manifestations noted by WHITFIELD were present, viz, (1) acute vesiculo-bullous manifestations with the characteristics of vesicular eczema; (2) chronic intertiginous manifestations, characterized by whitened, sodden masses of epithelium between the toes; (3) chronic hyperkeratotic manifestations characterized by large and irregular masses of overgrown horny layer. All these manifestations are usually accompanied by itching.

The difficulties encountered in making a diagnosis of dermatophytosis of the feet from the appearance of the lesions alone are great, and this is especially true of the intertriginous type. In this study a definite diagnosis of dermatophytosis of the feet was made only when pathogenic fungi were found either by microscopic examination or by culture.

Age. — The average age of the plate printers and rotary press printers was 52.6 years and 17.1 per cent of the plate printers were 60 years of age or older.

Industrial dermatitis of the hands and the past medical history of industrial dermatitis of the hands was approximately the same for each 10 year age group.

Duration of Employment. — Twenty-six and six-tenths per cent of the plate printers and rotary-press printers had been employed as such at the present establishment for 35 years or longer, while 74.5 per cent of the skilled helpers had been employed for 5 years or less.

All the plate printers who had dermatitis of the hands had worked at the present place of employment for 15 years or longer.

Section distribution. — Forty-two and eight-tenths per cent of the plate printers with dermatitis of the hands were working in sections 4 and 9 at the time of the examination. Only one plate printer developed dermatitis of the hands while employed in sections using black ink only, and his dermatitis was caused by cleaning his hands with sand. It is customary to transfer employees with dermatitis to the sections using only black ink.

Cleaning materials. — The cleaning materials, mineral oil, contaminated with various inks and debris, soaps, sand, salts of tartar, and brushes were found to be a definite factor in the causation of the dermatitis. Gasoline, which is used to clean the plates and the ink fountains, was also an etiological factor in the dermatitis.

Printing plates. — The type of plate was not found to be important in the production of dermatitis, except for the color of ink used thereon.

Inks. — Eleven of the thirteen iks used at this establishment caused dermatitis of the hands among the plate printers. The greatest offenders (listed in order of their frequency) were: orange No. 313, red No. 128, orange No. 311, and brown No. 513.

Family and personal histories. — The allergic disorders — hay fever, asthma, urticaria, migraine, and chronic eczema — occurred somewhat more frequently in the families of men with dermatitis than in the families of men who did not have dermatitis. The same relationship is true for the past personal histories of allergic disorders.

Date of onset of the dermatitis. — The incidence of dermatitis was approximately the same per year from 1900 through 1936.

Duration of the dermatitis of the hands. — From less than 1 year to more than 30 years.

Duration of dermatitis of the hands after leaving work. — Thirty-eight per cent and 56.7 per cent, respectively, of the employees with dermatitis of the hands, and employees who gave a history of dermatitis of the hands, had no loss of working time because of the dermatitis. The majority of the remaining affected employees recovered in from 1 week to 2 months after leaving work.

Estimate of the total loss of working time because of dermatitis of the hands. — Thirteen years working time for one man, during the period 1910 to 1936.

Seasonal exacerbation of the dermatitis. — None.

Inks used at onset of dermatitis. — Fifty-six and nine-tenths per cent of the plate printers, who either had a dermatitis of the hands, or a history of dermatitis of the hands, were working with green ink at the onset of the dermatitis, according to the medical history.

Length of time after beginning work at present establishment before dermatitis developed. — Approximately 50 percent of the employees had worked at present place for 10 years or longer before the dermatitis developed.

Description of the dermatitis. — Industrial dermatitis of the chronic eczematous type.

Mycological examination. — Pathogenic fungi of the feet were found in 33 percent by microscopic examination, 26.2 percent by culture, 36.4 per cent by one or both of these methods of examination. Thirty and five-tenths per cent revealed clinical evidence of dermatophytosis of the feet, and 49.7 per cent had either or both clinical evidence or laboratory findings of dermatophytosis of the feet. *Epidermophyton floccosum* was isolated from five of the men, *Trichophyton purpureum* from 18, and *T. mentagrophytes* from 71. The strains were isolated within a short period of time, and therefore provided exceptionally good material for a study of variations and species limits. Representative strains were selected, and comparative studies of strains were based on from 5 to 25 single spore isolates from each of 28 selected strains. A variety of culture types containing intergrading forms illustrates the variability of this species.

The controls, the plate printers with dermatitis of the hands, and the plate printers with a past history of dermatitis of the hands, had the same relative incidence of dermatophytosis of the feet by all of the above methods of examination.

No pathogenic fungi were found by culture or by microscopic examination on the hands.

Oil troughs. — The oil of the oil troughs did not possess fungicidal or bactericidal properties.

Shower baths. — Laboratory examination material taken from the shower bath floors revealed numerous nonpathogenic fungi, bacteria, and epithelial cells.

Patch tests. — Of the 121 individuals who were patch tested to the 13 inks used, 13 gave positive reactions. No positive tests were obtained among the 70 controls.

Fifty per cent of the plate printers who were patched, and who had dermatitis at the time of the examination, gave positive reactions to the inks.

Twelve and one-tenth per cent of the plate printers with a past history of dermatitis of the hands gave a positive reaction.

Results of the patch tests with component parts of inks were variable.

P. K. FRASER (1939) investigated some figures on the incidence of dermatophytosis, referring to previous statistical investigations carried out on this subject in the U. S. Navy by BUTLER (1924) (180 cases, 36 % positive), in the U. S. Army Reserve by WILSON (1934) (464 cases, 96.6 % positive), on one hand, and in student investigations by HULSEY and JORDAN (1925) (100 cases, 49 %), LEGGE et al. (1929) at the University of California (3,100 cases, 53.3 % positive in men, 15.3 % positive in women), GILMAN (1933) at the University of Philadelphia (785 cases, 60 % positive), ANDREWS and BIRKMAN (1931) at a New York trade school (520 cases, 12.5 % positive), MUSKATBLIT (1933) on patients of the Bellevue Hospital Skin Clinic and medical students (100 cases, 26 % positive), 112 cases, 32 % positive, respectively. On the other hand, — the author found on H. M. S. Hood in 1934 with an average complement of 1,120 individuals only 18 cases of mycotic infection, and in 1935 with an increased complement of 1,285 only 25 cases of dermatophytosis.

An associated tinea pedis and cruris, so commonly described by many writers was not seen in this series.

A case was regarded as clinically positive only if there was present a definite vesicular lesion on the foot.

In the 71 (16.6 %) clinically postive cases, there were 34 in the 4th decade, 32 in the 3rd decade, and 5 in the 2nd decade. 209 of the cases were of engineer branch and they had only 29 clinical cases of which 12 were confirmed microscopically. As a branch they work under conditions of heat and moisture with frequent washing in crowded bath spaces, all of which would seem ideal for fungus propagation, yet their positive rate was lower than any other branch in the series.

The author carried out another foot investigation on a more extensive scale during the autumn and winter of 1938 in small ships attached to the home stations. The pathogen was never recovered in the macerated type of lesion but was frequently found in feet showing only a tiny area of hyperkeratosis in the fourth interspace. 622 individuals were examined, form which 319 (51.3 %) were found with pedal lesions. Microscopically positive were only 33 (5.3 %). Among the total, there were 188 (30.2 %) individuals with previous mycotic history.

Of the 319 abnormal cases, only 23 showed no lesion in the fourth interspace, and apart from eczematoid areas on the dorsum, this was due to a malformation of the foot which caused cramping of the middle toes with an unduly open 4th interspace. 72.7 % of the „abnormal" cases (310) showed a closed or cramped type of foot and a similar condition was present in only 34.7 % of the normal cases (303).

Sixteen cases underwent a cultural examination. The following fungi were listed: *Tr. rubrum* (8×), *Tr. interdigitale* (2×), *Corethropsis hominis* (Vuill) (1×), *Epidermophyton floccosum* (3×). Beside these molds the cultures showed Staphylococci, and yeast, both or either of them. Two cases yielded only staphylococci.

W. ZUNDEL (1939) published a comprehensive survey on European *Epidermophyton* fungi. From 250 cases of Epidermophytosis of the Berlin Skin Clinic and Dispensary, he cultivated 133 Epidermophyton strains investigating them on a series of well known nutrient media as to their macroscopical and microscopical growth. In some strains the pathogenicity for laboratory animals was also investigated. All the strains, except for four of them with seemingly colony degeneration, could be arranged in three groups: (1) Fifteen strains revealing but unimportant differences among themselves were diaggnosed as *Epidermophyton inguinale*; (2) Sixty-eight strains showing fluent transitions in some of their important macroscopical and microscopical characteristics were, however, on the basis of numerous, typical features to be easily diagnosed, established as *Epidermophyton interdigitale* (Kaufmann-Wolf-fungus); (3) Forty-five strains showed radial furrows on Grutz' Nervina-malt nutrient media developing here and also on other nutrient media containing different carbohydrates a purple pigment. The pigment forming layer of the colony is lying directly in the medium as a tough continuous membrane. It is covered, starting from the center, in great variability with a white velvety mycelium or spores. Around the colony there was a loose cottony moist grayish yellow, or yellowish-green corona. The giant colonies recalled the pictures of *Ep. rubrum, Ep. plurizoniforme, Ep. lanoroseum,* and partly that of *Trichophyton pedis B* (Ota). All these strains were, however, despite their great variability in their pigment production, established as *Epidermophyton rubrum.*

The author critically surveyed the known *Epidermophyton fungi* pointing out the tendencies which emphasized the common characteristics of the variable *Epidermophyton* strains and required the establishment of basic types.

Epidermophyton rubrum was observed up to date very seldom and in few instances all over Europe. Present investigations revealed, however, that this fungus has at present a growing importance in the Berlin material, one third of all the cases of Epidermophytosis being caused by this fungus. The first place in frequency is still held by the *Epidermophyton interdigitale.*

N. F. Gavrilove investigated the epidemiology of epidermophytosis and the viability of *Epidermophyton fungi,* on dead vegetable material. Pinhead size material from a 30 day old cultures of *Trichophyton gypseum, Ep. inguinale,* and *Ep. Kaufmann-Wolf* was inoculated on different materials. The media were sterilized for 20 minutes at 120° C and before inoculation they were deposited on wet cotton sponges in broad test tubes. As material, birch, elm, pine, fir, and oak woods, rotten and painted woods, dung and soil were used. The inoculated material was kept for 10 days in the incubator at 27° C and further for 90 days at room temperature. Every 15 days, however, control inoculations were made on agar media to test the viability of the primary culture. All three species gave an equally good growth on the dead material than on agar media. *Ep. Kaufmann-Wolf* grew the most luxuriant producing strong swollen, gray-white colonies and only seldom the powdery yellowish variety when cultivated on dung. *Trichophyton gypseum* developed mainly powdery colonies and revealed a slower growth. A restricted growth was shown by the *Ep. inguinale,* the white colonies of which rarely revealed velvety surface. These fungi did not grow on fir and pine woods. The presence of resins in these woods accounts for that. The growth was, on the contrary, luxuriant on birch and elm woods.

Bertlich (1939) reported on fungus diseases in miners and their simple way of treatment. The author assumes that the disease is caused by an *Epidermophyton fungus,* above all, by *Ep. Kaufmann-Wolf.* The best therapeutic results were obtained by vioform-powder. The interdigital spaces of the toes were powdered and separated by a gauze strip from each other. The most severe cases were to be cured in three weeks. The results were not so good with another powder containing Sulf. precip. 5.0 and Zinc. oxyd., Talcum aa ad 200.0. The treatment with plain talcum powder was also unsatisfactory. Ninety patients were treated by means of vioform-powder. The author emphasized that under this treatment even the most severe cases retained their working ability and they became free of complaints after eight days. The good effect of the vioform-powder is traced to the structural place of iodine in the compound which permits killing the fungi without irritating the skin. A further advantage is seen in the chemical structure of the compound as a whole, which causes the restitution of the acid mantel of this skin disturbed by the mycosis.

P. Joulia and P. Lecoulant (1939) reported on generalized epidermomycoses of the late childhood caused by yeast-like fungi. The yeast infections of the late childhood take a middle place between the localized forms of the grown-ups and the generalized forms of the infancy. Their occurrence seems to be rare, there are only seven cases reported up to date in the literature. Based on their research in two cases and on their study of the literature, the authors differentiate two forms: one acute form corresponding to the disease in infancy and a chronic form with diffuse foci of different localization (skin, mucous membranes, nails, not involving the large skin folds) and causing sometimes visceral localization. They gave details of the clinical observation, the mycological investigation, the cultures (*Monilia albicans*) and the histological findings.

Riichi Kojima (1939) reported on Trichophytosis pompholyciformis with onychomycosis trichophytina in a little girl. Four months after birth the mother noticed some vesicles in the right palm of her daughter. The lesions spread and later on they attacked the nails. The right palm showed a diffuse redness with peripheral blisters. The normal luster of the nails of the right thumb, index and middle finger got lost, revealing an uneven, opaque surface. Mycelia were found in the roof of the blisters and in the deposits from the nail. The fungus was identified as *Trichophyton purpureum* Bang.

Sime Cajkovac (1939) investigated the frequency of the dyshidrosiform mycoses. From 1928 to 1937 inclusive, 405 cases of dyshidrosiform mycoses were observed in women. Monthly morbidity statistics showed a peak in the months of July and August suggesting a seasonal influence. Further investigations revealed, however, that this impression was untrue. The author rather explained the peak of morbidity in the two summer months as sequelae of sweating and frequent bathing which loosened the skin preparing the way for an infection. The dyshidrosiform mycoses must not be regarded as direct seasonal diseases depending on meteorological peculiarities. They are, however, somehow connected with atmospheric factors in certain seasons.

Allington (1939) observed hyperkeratotic fungous infection of the palms and soles. In one of the cases, a white man aged 29, revealed a fungous infection of the left hand, both feet and several nails for one year's duration. The involved areas are hyperkeratotic and show irregular ragged superficial scaling. An occasional deepseated vesicle is seen on the sides of the fingers or interdigital webs. The nail show hyperkeratosis of the nail bed with elevation of the nail plate first at the lateral and distal margins. Abundant mycelium has been repeatedly seen microscopically in horny material removed from the palms, soles and nails. The causative organism has not been identified culturally. The patient has been continuously treated with fungicidal preparation for the past nine months without any result. Similarly unsuccessful was the treatment in another patient, white man aged 33, presenting a fungous infection of the right palm and fingers, of the feet and several nails of three and onehalf year's duration. Mycelium was easily observed microscopically.

These two cases illustrate the point how superficially the diagnosis of fungous infections are made, simply on the basis of circumstantial evidences:

location of the lesions, a few or abundant mycelial threads in the scrapings under the microscope, without cultural proofs. Even a simple culture would reveal very often that the mycelial threads belong to no pathogenic fungi at all but to common molds from the genera *Penicillium, Aspergillus, Mucor,* etc. vegetating in the hyperkeratotic detritus masses. Moreover, real fungus infections of the nails are extremely rare. We know a few instances of nail infections by *Favus, Trichophyton.* The *Epidermophyton interdigitale,* very often found in detritus from the toes, has no etiologic significance at all. It is just as ubiquitous, just as harmless as the common molds mentioned above.

These two cases give the exact description of the chronic hyperkeratotic therapy-resistent type of pompholyx which is — so often emphasized by the reviewer — not an exogenous fungous infection but an endoparasitic, bloodborne affair. Hence the total failure of local application of fungicides.

HERMAN GOODMAN (1939) made observations on ringworm of the hand which indicate that the problem is not an easy one for solution. He illustrated the possible difficulties of an exact diagnosis by divers examples. In a case of a syphilitic gumma fungi were recovered from the lesion microscopically and macroscopically. The lesion promptly disappeared without any local therapy due to antisyphilitic treatment. In a number of workers in the silk industry, fungi were found in the lesions of the hands. Thorough investigation revealed that the lesions were caused by certain chemicals used in the factory. Fungi as secondary invaders need more attention. The author points out the unspecific diagnostic and therapeutic effect of Trichophytin.

Deficiencias Vitaminicas de Cogumelos Patogenicos

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O estudo das exigencias vitaminicas dos cogumelos patogenicos é assunto que só recentemente tem recebido a devida atenção da parte dos diversos pesquizadores. Apesar de varias serem as publicações isoladas escritas sobre a materia, poucos são os trabalhos de conjunto até então publicados, contando-se principalmente dentre estes, o de Oyama (1937), um trabalho de revisão de Robbins & Kavanagh (1942), sobre exigencias vitaminicas de cogumelos de um modo geral, compreendendo especies patogenicas e não patogenicas e o trabalho de Burkholder & Moyer (1943), onde tambem são estudadas as deficiencias vitaminicas de certo numero de cogumelos patogenicos. Os dados acumulados sobre o assunto, são muito bem sumariados no trabalho de Nickerson & Williams (1947). Contudo, na literatura por nós consultada, não encontramos referencia a trabalho experimental onde fossem estudadas em conjunto es exigencias vitaminicas dos cogumelos patogenicos.

No presente trabalho estudamos as deficiencias vitaminicas de 60 amostras de cogumelos pertencentes a 50 especies e a 31 generos diferentes. Evidentemente os resultados obtidos só serão considerados validos para as amostras estudadas e nas condições da nossa experimentação, não se podendo aplica-los às diversas especies, dada a sua variabilidade não só morfologica mas tambem fisiologica. Fazemos um trabalho de conjunto, um ensaio preliminar, servindo para a seleção de material destinado a estudos posteriores mais detalhados sobre cada genero, especie ou amostra.

Conforme se verá a seguir, só estudamos cogumelos patogenicos ou como tal considerados, isto é, cogumelos que tenham sido isolados de processos varios e pelos mesmos responsabilizados. A proposito de cada caso particular, daremos as referencias bibliograficas que pudemos consultar, diretamente relacionadas ao assunto, sem pretendermos contudo ter revisto toda a bibliografia.

Parte experimental

Material e metodos

Meio de cultura: Em todas as nossas experiencias usamos meio de cultura basico semelhante ao utilizado por Robbins (1939 a), cuja composição é a seguinte:

Dextrose anidra (Merck)	30.0 gr.
l (+) asparagina (Pfanstiehl)	2.0 gr.
KH_2PO_4	1.5 gr.
$MgSO_4.7H_2O$	0.5 gr.
Agar	12.0 gr.
Agua distilada q.s.	1.000.0 ml.

A 1.000 ml da solução acima, juntar 0.1 ml da seguinte solução mineral:

H_3BO_3	5.7 mgr.
$CuSO_4.5H_2O$	18.6 mgr.

$Fe(NH_4)_2.(SO_4)_2.6H_2O$	173.0 mgr.
$MnSO_4.4H_2O$	8.1 mgr.
$(NH_4)_6.MoO_{24}.4H_2O$	3.6 mgr.
$ZnSO_4.7H_2O$	79.0 mgr.
Agua distilada q.s.	100.0 ml.

O pH do meio foi acertado a 5.5 com solução de NaOH 2N.

A asparagina foi purificada por duas recristalizações sucessivas. A tecnica usada para a sua purificação foi a seguinte: dissolver a substancia nagua (solubilidade 1 : 47 a 20° C) e adicionar acido acetico glacial (1 : 1000). Juntar carvão ativado (20 gr. por litro) e agitar durante 1 hora. Filtrar em papel. Adicionar alcool etilico absoluto (3 vezes o volume da solução aquosa) e colocar em refrigerador, onde permanece por 24 horas. A asparagina precipita por ser insoluvel em alcool. Filtrar em Buchner e lavar com varias porções de alcool absoluto frio. Secar a 45—50° C.

Tambem o agar foi purificado afim de se eliminar os fatores de crescimento acompanhantes. Varias são as tecnicas aconselhadas e usadas para a sua purificação (Robbins, 1939 b; Robbins & Ma, 1941; Day, 1942; Ryan, Beadle & Tatum, 1943), mas todas elas obedecem praticamente à mesma sequencia. Usamos a tecnica de Robbins & Ma (1941) procedendo do seguinte modo: tratar 100 gramas de agar granulado ,,Difco'' por 3 litros de solução aquosa de piridina a 5 %, deixando o agar imerso nessa solução durante 24 horas, agitando-se de vez em quando. No fim desse periodo, eliminar o maximo possivel da solução aquosa. Devido à imbibição do agar em agua, torna-se dificil elimina-la completamente por este processo, o que todavia se consegue pela adição de alcool etilico absoluto em grande volume (5 litros). Misturar bem e filtrar em Buchner, com aspiração. Retomar o agar com 3 litros de alcool absoluto e ferver por 3 a 4 horas. Filtrar em Buchner e repetir nova extração a quente. Filtrar novamente até que se tenha eliminado o maximo possivel de liquido. Lavar o agar ainda no Buchner com varias porções de alcool absoluto. Secar a 50—60° C.

As vitaminas utilizadas nos ensaios foram as seguintes: tiamina (cloridrato), riboflavina, piridoxina (cloridrato), acido nicotinico, pantotenato de calcio, inositol e biotina. As soluções dessas vitaminas foram preparadas separadamente e mantidas em refrigerador. Sua adição ao meio basico foi feita de modo a se ter a seguinte concentração final por litro de meio: tiamina 500 μg, riboflavina 500 μg, piridoxina 500 μg, acido nicotinico 500 μg, pantotenato de calcio 500 μg, inositol 10.000 μg (10 mgr.) e biotina 5 μg.

Ao todo preparamos 10 diferentes tipos de meio, a saber: meio basico sem vitaminas (*meio* 1); meio basico contendo as sete vitaminas (*meio* 9); meio basico contendo as sete vitaminas e 1 % de extrato de levedura ,,Difco'' (*meio* 10); os sete meios restantes foram preparados de modo a conter, cada um, 6 vitaminas, isto é, omitindo-se uma vitamina dentre as sete usadas, nos meios de nos. 2 a 8, na seguinte ordem: (*meio* 2) tiamina; (*meio* 3) riboflavina; (*meio* 4) piridoxina; (*meio* 5) acido nicotinico; (*meio* 6) pantotenato de calcio; (*meio* 7) inositol; (*meio* 8) biotina.

Uma vez misturados e dissolvidos os componentes dos meios, foram os mesmos distribuidos em tubos de 18 × 180 mm, em quantidades de 10 ml por tubo e autoclavados a 120° C durante 20 minutos.

Vidraria — Os tubos de cultura empregados eram de vidro neutro. A limpesa da vidraria mereceu cuidados especiais. Os tubos eram mergulhados em mistura sulfo-cromica e aí deixados durante 24 horas, após o que eram exaustivamente lavados e secos.

Culturas — As culturas por nós utilizadas na experimentação são todas da Coleção da Secção de Micologia do Instituto Oswaldo Cruz. A nomenclatura tal como é empregada no presente trabalho, corresponde à original, isto é, usada tal como nos foi enviada. O numero que se segue após o nome de cada especie corresponde ao numero que as mesmas teem em nossa Coleção. Damos a seguir a relaçãodas culturas estudadas com a respectiva proveniencia e a data em que as mesmas foram incluidas na Coleção.

Achorion gallinae 1456 — Recebida de Negroni com o no. 42. (1930).
Achorion gypseum 1439 — Recebida de Negroni com o no. 32. (1930).
Achorion Schoenleinii 1425 — Recebida de Negroni com o no. 19. (1930).
Acremoniella Lutzi 866 — Isolada de lesão ossea (peroneo). Amostra original (Area Leao & Lobo, 1939). (1939).
Acremonium Potronii 1610 — Isolada de lesão localizada no joelho. Primeiro caso de acremoniose observado no Brasil (Gonzaga & Area Leao, 1934). (1933).
Actinomyces brasiliensis 2590 — Recebida de Lacaz com o numero 5. (1948).
Actinomyces madurae 2593 — Recebida de Lacaz com o no. 43. (1948).
Allescheria Boydii 386— Amostra original de Shear. (1921).
Allescheria Boydii 1699 — Recebida de Negroni com o numero 25 e com a indicação de ter sido recebida do laboratorio de Thom, U.S.D.A. (1934).
Blastomyces dermatitidis 1901 — Recebida de Conant com o no. 836 (1945).
Blastomyces dermatitidis 1902 — Recebida de Conant com o no. 835 (1945).

Candida suaveolens 2032 — Recebida de Mackinnon com o no. 752. Trata-se da amostra de *Neogeotrichum pulmoneum* original de Magalhaes (1932), identificada a *Trichosporon cutaneum* por Puntoni (1938) e a *Candida suaveolens* por Mackinnon & A. Allende (1945) e Mackinnon (1946). (1948).

Cephalosporium recifei 1669 — Isolada de um caso de micetoma de grãos brancos. Amostra original (Area Leao & Lobo, 1934). (1934).

Cladosporium Wernecki 1886 C —

Cladosporium Wernecki 1886 M — Isolada de um caso de ,,tinea nigra" (,,Keratomycosis nigricans palmaris"). Foi observado (Cury, 1944) que esta amostra apresentava o ,,dual phenomenon" (Hansen, 1938). Desde o seu isolamento são mantidos na Coleção os dois tipos, ,,M" (,,mycelial") e ,,C" (,,conidial"). (1945).

Coccidioides immitis 1893 — Recebida de Conant com o no. 794. (1945).

Coccidioides immitis 1894 — Recebida de Conant com o no. 798. (1945).

Coccidioides immitis 1897 — Recebida de Conant com o no. 819. (1945).

Debaryomyces Hudeloi 1940 — Recebida de Negroni. (1945).

Endodermophyton concentricum 1176 — Recebida de Ota, Faculdade de Medicina, Universidade Imperial de Tohoku, Senday, Japão, com o no. 256. (1928).

Endodermophyton Roquettei 1061 — Isolada de um caso de ,,Chimbere", dermatose escamosa generalizada dos indios do Rio São Miguel. Amostra original (Fonseca, 1925). (1925).

Endodermophyton tropicale 178 — Recebida de Negroni com o no. 163. (1934).

Epidermophyton floccosum 1923 — Isolada de escamas dos espaços interdigitais. (1945).

Glenosporella Loboi 1488 — Isolada de um caso de blastomicose queloideana (doença de Jorge Lobo). Amostra original (Fonseca & Area Leao, 1940).

Glenosporopsis amazonica 1848 — Isolada de nodulos duros e salientes localizados proximos ao olecraneo. Amostra original (Fonseca, 1943).

Haplosporangium parvum 2606 — Recebida de Conant. (1948).

Hemispora stellata 1125 — Isolada de lesão cutanea interdigital. (1927).

Histoplasma capsulatum 954 — Recebida do Dr. H. E. Meleney, Vanderbildt University, com a nota ,,Dog strain". (1940).

Histoplasma capsulatum 1860 — Recebida de Negroni com o no. 922. E a amostra isolada por Negroni de um caso de histoplasmose observado em Buenos Aires. (1944).

Histoplasma capsulatum 2357 — Recebida de Madureira Pará. Isolada de cão relacionado ao segundo caso humano de histoplasmose observado no Brasil, achado por viscerotomia (Para, 1946). (1947).

Hormiscium dermatitidis 1800 — Recebido de Ota, com a nota: ,,499. *Hormiscium dermatitidis* Kano, 1934".

Hormodendrum algeriensis 2222 — Recebida de Negroni com o no. 915. (1947).

Hormodendrum Langeroni 990 — Isolada de lesões acromicas da pele. (1945).

Madurella americana 1182 — Recebida de Ota. (1928).

Madurella Ikedai 2583 — Recebida do Centraalbureau voor Schimmelcultures, Baarn, Holanda. (1948).

Madurella mycetomi 2372 — Recebida de Negroni com o no. 942. (1947).

Malassezia tropica 2430 — Recebida da American Type Culture Collection, com o no. 7123. (1947).

Microsporum Audouini 1447 — Recebida de Negroni com o no. 10. (1930).

Microsporum canis 2555 — Isolada de placa de tinha tonsurante de uma criança de 6 anos. (1948).

Microsporum ferrugineum 569 — Recebida de Ota, Mandchuria. (1921).

Monosporium apiospermum 1603 — Proveniente do Instituto Botanico, Laboratorio Crittogamico, R. Universitá di Pavia. (1933).

Paracoccidioides brasiliensis 2594 — Recebida de Lacaz; isolada do doente J.A. (1948).

Paracoccidioides brasiliensis 2596 — Idem, com a nota ,,Madeira". (1948).

Paracoccidioides brasiliensis 2597 — Idem, isolada do doente L. M. (1948).

Phialophora compactum 1991 — Recebida de Emmons com a nota: ,,8616, *Phialophora compactum* (col. L. Ferraro)." (1945).

Phialophora Jeanselmei 2000 — Recebida de Emmons com a nota: ,,8721, *Phialophora Jeanselmei* (Langeron) Emmons. (*Torula Jeanselmei* Langeron), from a mycetoma of hand". (1945).

Phialophora Pedrosoi 1885 — Isolada de um caso de cromoblastomicose. Lesões localizada no pé. (1944).

Phialophora verrucosa 1995 — Recebida de Emmons com a nota: ,,8704, *P. verrucosa* (*Cadophora americana* from wood). (1945).

Piedraia Hortai 1538 — Isolada de um caso de piedra negra em criança de 12 anos. (1931).

Piedraia Hortai 2458 — Isolada de um caso de piedra negra em adulto. (1947).

Sporotrichum Schenckii 2562 — Isolada de lesões fechadas localizadas na mão e antebraço. (1948).

Torulopsis neoformans 2561 — Proveniente do Centraalbureau voor Schimmelcultures. (1948).
Trichophyton mentagrophytes 2543 — Isolada de lesões dos pés, tipo pé de atleta. (1947).
Trichophyton rubrum 2553 — Isolada de lesões do torax e braço. (1948).
Trichophyton Sabouraudi 512 — Isolada por O. da Fonseca na clinica de Sabouraud (Hospital St. Louis, Paris). (1921).
Trichophyton sulfureum 1421 — Recebida de Negroni com o no. 100 (1930).
Trichophyton tonsurans 612 — Isolada do caso 67 (O. da Fonseca). (1921).
Trichophyton violaceum 1254 — Isolada de placa de tricoficea seca do couro cabeludo. (1929).
Trichosporon Beigelii 2041 — Recebida de Negroni. (1945).
Trichosporon minor 984 — Isolada de piedra axilar. Amostra original, (Arêa Leão, 1940). (1940).

Inoculum — As culturas antes de serem semeadas nos varios tipos de meio foram previamente transplantadas para um meio contendo 1 % de bacto-peptona, 3 % de dextrose, 1 % de extrato de malte ,,Difco'' e 1.5 % de bacto-agar, onde as mesmas se desenvolviam por um periodo de cerca de 30 dias, após o que procediamos ao inoculum, feito de modo a se transplantar para os meios de prova um fragmento da cultura tão pequeno quanto possivel, de preferencia retirando-o da porção aerea da cultura, evitando-se tocar o meio ou levar conjuntamente pequenas porções do mesmo o que importaria em transportar os fatores de crescimento presentes no meio original. Nas culturas que se desenvolviam muito aderentes ao meio, sem formação de micelio aereo, mesmo raso, dificeis de se destacar, ou se destacando às vezes completamente, porções maiores eram retiradas e seccionadas com o auxilio de uma pequena espatula de platina em pequenos fragmentos os quais eram lavados repetidas vezes em agua distilada esteril e em seguida plantados.

Os tubos, uma vez semeados, eram deixados à temperatura ambiente. As observações do crescimento foram feitas com intervalos de 10—15 dias, até um periodo total de 50—60 dias, findo o qual as culturas eram fotografadas. Os principais detalhes sobre o seu aspeto macroscopico foram anotados no decorrer da experiencia.

Indicamos por — a ausencia de crescimento ou crescimento muito reduzido; por +, ++, +++, ++++ as intensidades crescentes do desenvolvimento, tomando-se sempre o crescimento no meio com extrato de levedura como ++++.

Resultados — Os resultados obtidos nessas condições podem ser melhor observados na Tabela I, bem como nas fotografias (Figuras 1 a 58). Contudo, relataremos a seguir os dados colhidos da observação macroscopica das culturas estudadas. Não cogitamos no presente trabalho dos detalhes da sua observação microscopica nos diferentes meios utilizados.

Achorion gallinae 1456 — Apresentou bom desenvolvimento em todos os meios, mais intenso porem no meio contendo extrato de levedura que exerce ação estimulante. Não apresentou exigencias para as vitaminas ensaiadas. (Fig. 1).

Achorion gypseum 1439 — Bom desenvolvimento em todos os meios, sendo mais intenso no meio contendo extrato de levedura que tem ação estimulante. Não exige as vitaminas ensaiadas. (Fig. 2).

Achorion Schoenleinii 1425 — Crescimento nulo no meio basico e muito reduzido nos meios de 2 a 9; abundante no meio com extrato de levedura que tem intensa ação estimulante do crescimento. Não mostrou exigencias para as vitaminas usadas. (Fig. 3).

Acremoniella Lutzi 866 — Desenvolvimento abundante em todos os meios. Não exigente. Extrato de levedura sem ação estimulante visivel. (Fig. 4).

Acremonium Potronii 1610 — Crescimento nulo nos meios 1 e 2; abundante nos demais. Produção de pigmento pardo que se difunde para o meio; formação de goticulas na superficie da cultura. Extrato de levedura sem ação estimulante visivel. (Fig. 5). Exige tiamina.

Actinomyces brasiliensis 2590 — Bom desenvolvimento em todos os meios, mais intenso porem no meio contendo extrato de levedura que tem ação estimulante. Não exige as vitaminas usadas. (Fig. 6).

Actinomyces madurae 2593 — Crescimento moderado nos meios de 1 a 9; bom desenvolvimento no meio 10. Não exige as vitaminas ensaiadas. Nitida ação estimulante do extrato de levedura. (Fig. 7).

Allescheria Boydii 386 — Desenvolvimento nulo nos meios 1 e 8; bom nos demais. Exige biotina. Extrato de levedura sem ação estimulante apreciavel. (Fig. 8).

Allescheria Boydii 1699 — Crescimento nulo nos meios 1 e 8; bom nos demais. O extrato de levedura tem ligeira ação estimulante. Produção de pigmento violaceo na superficie da cultura, não se difundindo para o meio. Exige biotina. (Fig. 9).

Blastomyces dermatitidis 1901 — Bom crescimento em todos os meios. Discreta ação estimulante do extrato de levedura. Não exigente. (Fig. 10).

Blastomyces dermatitidis 1902 — Crescimento moderado nos meios de 1 a 9; mais abundante no meio 10. Não exigente. Ação estimulante do extrato de levedura. (Fig. 11).

Candida suaveolens 2032 — Crescimento nulo nos meios 1 e 2. Bom desenvolvimento nos demais, sendo mais abundante no meio contendo extrato de levedura que exerce uma nitida ação estimulante. Deficiente para tiamina. (Fig. 12).

Cephalosporium recifei 1669 — Bom crescimento em todos os meios. Não exigente. Extrato de levedura sem ação estimulante apreciavel.

Cladosporium Wernecki 1886 C — Bom desenvolvimento em todos os meios. Não exigente. Extrato de levedura sem ação apreciavel. (Fig. 13).

Cladosporium Wernecki 1886 M — Bom desenvolvimento em todos os meios. Aspeto aveludado e cor oliva-escura nos meios de 1 a 9; no meio 10 a colonia é ûmida e negra. Extrato de levedura sem ação estimulante. Não exigente. (Fig. 14).

Coccidioides immitis 1893 — Crescimento regular nos meios de 1 a 9; abundante no meio 10. Não exigente. O extrato de levedura exerce nitida ação estimulante. (Fig. 15).

Coccidioides immitis 1894 — Desenvolvimento regular nos meios de 1 a 9; abundante no meio 10. Não exigente para as vitaminas ensaiadas. O extrato de levedura exerce nitida ação estimulante. (Fig. 16).

Coccidioides immitis 1897 — Crescimento reduzido nos meios de 1 a 9; bom no meio 10. Não exigente. Nitida ação estimulante do extrato de levedura. (Fig. 17).

Debaryomyces Hudeloi 1940 — Desenvolvimento nulo nos meios 1 e 8; bom nos demais, sendo mais abundante no meio contendo extrato de levedura que tem nitida ação estimulante do crescimento. Deficiente para biotina. (Fig. 18).

Endodermophyton concentricum 1176 — Bom desenvolvimento em todos os meios. Não exigente. O extrato de levedura tem ligeira ação estimulante. (Fig. 19).

Endodermophyton Roquettei 1061 — Bom crescimento em todos os meios, sendo mais abundante no meio contendo extrato de levedura, que tem ação estimulante. Não exigente. (Fig. 20).

Endodermophyton tropicale 1718 — Bom desenvolvimento nos varios meios, sendo mais abundante no meio contendo extrato de levedura, que tem moderada ação estimulante. Não exigente. (Fig. 21).

Epidermophyton floccosum 1923 — Bom desenvolvimento em todos os meios, sendo mais abundante no meio contendo extrato de levedura que tem ação favorecedora do crescimento. Produção de pigmento amarelado que difunde para o meio. Não exigente. (Fig. 22).

Glenosporella Loboi 1488 — Bom crescimento nos meios de 1 a 9, sendo um pouco maior no meio contendo extrato de levedura que mostra ação estimulante. Não exigente. (Fig. 23).

Glenosporopsis amazonica 1848 — Crescimento nulo nos meios 1 e 2; bom nos demais. Extrato de levedura sem ação estimulante apreciavel. Deficiente para tiamina. (Fig. 24).

Haplosporangium parvum 2606 — Bom desenvolvimento em todos os meios, mais abundante no meio contendo extrato de levedura que exerce ação estimulante. Não exigente. (Fig. 25).

Hemispora stellata 1125 — Moderado desenvolvimento nos meios de 1 a 9; mais abundante no meio 10. Extrato de levedura tem ação estimulante. Não exigente. (Fig. 26).

Histoplasma capsulatum 954 — Bom crescimento em todos os meios. Não exigente. O extrato de levedura tem ação estimulante discreta. (Fig. 27).

Histoplasma capsulatum 1860 — Bom desenvolvimento em todos os meios, sendo um pouco maior no meio 10. O extrato de levedura exerce ligeira ação estimulante. Não exigente. (Fig. 28).

Histoplasma capsulatum 2357 — Crescimento nulo nos meios 1 e 2. Bom nos demais, sendo mais abundante no meio 10, onde se observa hifas aereas, ao contrario dos outros meios onde o micelio é raso e úmido. Ação estimulante do extrado de levedura. Exige tiamina. (Fig. 29).

Hormiscium dermatitidis 1800 — Crescimento praticamente nulo nos meios 1 e 2 (micelio submerso). Extrato de levedura sem ação estimulante appreciavel. Deficiente para tiamina. (Fig. 30).

Hormodendrum algeriensis 2222 — Crescimento quasi nulo nos meios 1 e 2; bom nos demais. Extrato de levedura sem ação estimulante visivel. Exige tiamina. (Fig. 31).

Hormodendrum Langeroni 990 — Bom desenvolvimento em todos os meios. Extrato de levedura sem ação estimulante visivel. Não exigente. (Fig. 32).

Madurella americana 1182 — Desenvolvimento abundante em todos os meios. Não exigente. Extrato de levedura sem ação estimulante. (Fig. 33).

Madurella Ikedai 2583 — No meio basico o desenvolvimento é mais reduzido que nos demais. Nos meios de 1 a 9, o bordo da colonia é de aspeto seco e aveludado, de cor creme-clara, com o centro mais elevado, pregueado, negro e de aspeto úmido. No meio contendo extrato de levedura, toda a colonia é de aspeto seco e aveludado, de coloração creme, sem qualquer ponto negro no centro. Intensa elaboração de pigmento pardo-escuro que se difunde para o meio. A mistura das vitaminas utilizadas parece ter ação estimulante em relação ao meio basico, o mesmo se dando com o extrato de levedura. (Fig. 34).

Madurella mycetomi 2372 — Desenvolvimento abundante em todos os meios. Não exigente. Extrato de levedura sem ação estimulante. (Fig. 35).

Microsporum Audouini 1447 — Crescimento praticamente nulo no meio basico e muito reduzido no meio 5; bom nos demais, sendo mais intenso no meio contendo extrato de levedura, o qual exerce ação estimulante. Deficiente para acido nicotinico. (Fig. 37).

Microsporum canis 2555 — Bom desenvolvimento em todos os meios. Não exigente. Ligeira ação estimulante do extrato de levedura. (Fig. 38).

Microsporum ferrugineum 569 — Crescimento muito reduzido nos meios de 1 a 9; abundante no meio contendo extrato de levedura o qual exerce uma intensa ação estimulante do crescimento. Não mostrou deficiencia para as vitaminas ensaiadas. (Fig. 39).

Monosporium apiospermum 1603 — Desenvolvimento abundante em todos os meios. Não exigente. Extrato de levedura sem ação estimulante. (Fig. 40).

Paracoccicioides brasiliensis 2594 — Desenvolvimento praticamente nulo nos meios 1 e 2; bom nos demais, sendo mais abundante no meio 10. Deficiente para tiamina. O extrato de levedura exerce ação estimulante.

Paracoccidioides brasiliensis 2596 — Desenvolvimento muito reduzido nos meios 1 e 2. Bom nos demais, sendo mais abundante no meio contendo extrato de levedura que tem ação estimulante. Deficiente para tiamina. (Fig. 41).

Paracoccidioides brasiliensis 2597 — Crescimento moderado nos meios 1 e 2; bom nos demais. O extrato de levedura tem ação estimulante discreta. Parcialmente deficiente para tiamina. (Fig. 42).

Phialophora compactum 1991 — Crescimento muito reduzido nos meios 1 e 2 (micelio submerso); bom nos demais. Extrato de levedura sem ação estimulante apreciavel. Exige tiamina. (Fig. 43).

Phialophora Jeanselmei 2000 — Crescimento praticamente nulo nos meios 1 e 2 (micelio submerso); bom nos demais. Extrato de levedura sem ação estimulante apreciavel. Deficiente para tiamina. (Fig. 44).

Phialophora Pedrosoi 1885 — Crescimento muito reduzido nos meios 1 e 2 (micelio submerso); bom nos demais. O extrato de levedura não tem ação estimulante. Deficiente para tiamina. (Fig. 45).

Phialophora verrucosa 1995 — Desenvolvimento quasi nulo nos meios 1 e 2 (micelio submerso); bom nos demais. O extrato de levedura não mostra ação estimulante. Exige tiamina. (Fig. 46).

Piedraia Hortai 1538 — Crescimento nulo nos meios 1 e 2; moderado nos demais, sendo mais abundante no meio contendo extrato de levedura o qual tem uma nitida ação estimulante do crescimento. Produção de pigmento escuro que se difunde para o meio. Deficiente para tiamina. (Fig. 47).

Piedraia Hortai 2458 — Crescimento praticamente nulo nos meios 1 e 2; moderado nos demais, sendo mais abundante no meio 10. A produção de pigmento é menos intensa que no caso anterior. O extrato de levedura tem nitida ação estimulante do crescimento. Exige tiamina. (Fig. 48).

Sporotrichum Schenckii 2562 — Crescimento nulo nos meios 1 e 2; bom nos demais, sendo mais abundante no meio 10. O extrato de levedura tem ação estimulante do crescimento. Deficiente para tiamina. (Fig. 49).

Torulopsis neoformans 2561 — Crescimento nulo nos meios 1 e 2; abundante nos demais. Não foi observada ação favorecedora do extrato de levedura. Exige tiamina. (Fig. 50).

Trichophyton mentagrophytes 2543 — Crescimento reduzido nos meios 1, 2 e 7; moderado nos demais e abundante no meio 10. O extrato de levedura tem nitida ação estimulante. Deficiente para tiamina e inositol. (Fig. 51).

Trichophyton rubrum 2553 — Bom desenvolvimento nos meios de 1 a 9; mais abundante no meio contendo extrato de levedura onde tambem a produção de pigmento é mais intensa que nos demais, sendo que no meio 4 a produção de pigmento é muito reduzida, praticamente nula. Não exige as vitaminas ensaiadas. Ligeira ação estimulante do extrato de levedura. (Fig. 52).

Trichophyton Sabouraudi 512 — Bom desenvolvimento nos meios de 1 a 9; mais abundante no meio contendo extrato de levedura que tem nitida ação estimulante do crescimento. Não exigente. (Fig. 53).

Trichophyton sulfureum 1421 — Desenvolvimento nulo nos meios de 1 a 9; (reduzido ao inoculum). Bom no meio contendo extrato de levedura que mostra uma intensa ação estimulante do crescimento. Não exige as vitaminas ensaiadas. (Fig. 54).

Trichophyton tonsurans 612 — Desenvolvimento reduzido nos meios de 1 a 9; abundante no meio 10. Não exigente. O extrato de levedura exerce intensa ação favorecedora do crescimento. (Fig. 55).

Trichophyton violaceum 1254 — Desenvolvimento nulo nos meios de 1 a 9 (reduzido ao inoculum); abundante no meio 10. Não exige as vitaminas ensaiadas. O extrato de levedura exerce intensa ação favorecedora do crescimento. (Fig. 56).

Trichosporon Beigelii 2041 — Desenvolvimento praticamente nulo nos meios 1, 2 e 8; bom nos demais. Deficiente para tiamina e biotina. Não foi observada ação estimulante do extrato de levedura. (Fig. 57).

Trichosporon minor 984 — Desenvolvimento praticamente nulo nos meios 1 e 2; bom nos demais. Exige tiamina. Extrato de levedura sem ação estimulante apreciavel. (Fig. 58).

Discussão

De acordo com os resultados obtidos, verifica-se que das 60 amostras estudadas, 35 não mostram exigencias para as vitaminas ensaiadas: *Achorion gallinae, A. gypseum, A. Schoenleinii, Acremoniella Lutzi, Actinomyces brasiliensis, A. madurae, Blastomyces dermatitidis* (2 amostras), *Cephalosporium recifei, Cladosporium Wernecki* 1886 C e 1886 M, *Coccidioides immitis* (3 amostras), *Endodermophyton concentricum, E. Roquettei, E. tropicale, Epidermophyton floccosum, Glenosporella Loboi, Haplosporangium parvum, Hemispora stellata, Histoplasma capsulatum* (2 amostras), *Hormodendrum Langeroni, Madurella americana, M. mycetomi, Malassezia tropica, Microsporum canis, M. ferrugineum, Monosporium apiospermum, Trichophyton rubrum, T. Sabouraudi, T. sulfureum, T. tonsurans* e *T. violaceum.*

As 25 amostras que mostraram exigencias para uma ou mais das vitaminas ensaiadas (auxo-heterotroficas), podem ser assim distribuidas:

a) A mistura das vitaminas parece ter uma ação estimulante sobre o crescimento, em relação ao meio basico — 1 amostra (*Madurella Ikedai*).

b) Totalmente deficientes para tiamina — 17 amostras (*Acremonium Potronii, Candida suaveolens, Glenosporopsis amazonica, Histoplasma capsulatum* 2357, *Hormiscium dermatitidis, Hormodendrum algeriensis, Paracoccidioides brasiliensis* 2594, *P. brasiliensis* 2596, *Phialophora compactum, P. Jeanselmei, P. Pedrosoi, P. verrucosa, Piedraia Hortai* 1538, *P. Hortai* 2458, *Sporotrichum Schenckii, Torulopsis neoformans* e *Trichosporon minor*).

c) Parcialmente deficiente para tiamina — 1 amostra (*Paracoccidiodes brasiliensis* 2597).

d) Deficientes para biotina — 3 amostras (*Allescheria Boydii* 386, *A. Boydii* 1699 e *Debaryomyces Hudeloi*).

e) Deficiente para acido nicotinico — 1 amostra (*Microsporum Audouini*).

f) Deficiente para tiamina e biotina — 1 amostra *Trichosporon Beigelii*).

g) Deficiente para tiamina e inositol — 1 amostra (*Trichophyton mentagrophytes*).

TABLE I

Vitamin requirements of pathogenic fungi grown in a basal mineral medium containing dextrose and asparagin with varied supplements of vitamins and yeast extract.

ORGANISM	1 Basal medium without vitamins	2 — Thiamin	3 — Riboflavin	4 — Pyridoxin	5 — Nicotinic acid	6 — Calcium pantothenate	7 — Inositol	8 — Biotin	9 + All vitamins	10 + All vitamins and 1% yeast extract	VITAMIN REQUIREMENTS	GROWTH IN YEAST EXTRACT
Achorion gallinae 1456	+++	+++	+++	+++	+++	+++	+++	+++	+++	++++	No deficiency observed	Stimulated
Achorion gypseum 1439	+++	+++	+++	+++	+++	+++	+++	+++	+++	++++	No deficiency observed	Stimulated
Achorion Schoenleinii 1425	-	+	+	+	+	+	+	+	+	++++	No deficiency observed	Greatly stimulated
Acremoniella Lutzi 866	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	No deficiency observed	Not stimulated
Acremonium Potronii 1610	-	-	++++	++++	++++	++++	++++	++++	++++	++++	Thiamin	Not stimulated
Actinomyces brasiliensis 2590	+++	+++	+++	+++	+++	+++	+++	+++	+++	++++	No deficiency observed	Stimulated
Actinomyces madurae 2593	++	++	++	++	++	++	++	++	++	++++	No deficiency observed	Stimulated
Allescheria Boydii 386	-	++++	++++	++++	++++	++++	++++	-	++++	++++	Biotin	Not stimulated
Allescheria Boydii 1699	-	+++	+++	+++	+++	+++	+++	-	+++	++++	Biotin	Stimulated
Blastomyces dermatitidis 1901	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	No deficiency observed	Somewhat stimulated
Blastomyces dermatitidis 1902	+++	+++	+++	+++	+++	+++	+++	+++	+++	++++	No deficiency observed	Stimulated
Candida suaveolens 2032	-	-	+++	+++	+++	+++	+++	+++	+++	++++	Thiamin	Stimulated
Cephalosporium recifei 1669	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	No deficiency observed	Not stimulated
Cladosporium Wernecki 1886 C	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	No deficiency observed	Not stimulated
Cladosporium Wernecki 1886 M	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	No deficiency observed	Not stimulated
Coccidioides immitis 1893	++	++	++	++	++	++	++	++	++	++++	No deficiency observed	Stimulated
Coccidioides immitis 1894	++	++	++	++	++	++	++	++	++	++++	No deficiency observed	Stimulated
Coccidioides immitis 1897	++	++	++	++	++	++	++	++	++	++++	No deficiency observed	Stimulated
Debaryomyces Hudeloi 1940	-	++	++	++	++	++	++	-	++	++++	Biotin	Stimulated
Endodermophyton concentricum 1176	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	No deficiency observed	Somewhat stimulated
Endodermophyton Roquettei 1061	+++	+++	+++	+++	+++	+++	+++	+++	+++	++++	No deficiency observed	Stimulated
Endodermophyton tropicale 1718	+++	+++	+++	+++	+++	+++	+++	+++	+++	++++	No deficiency observed	Stimulated
Epidermophyton floccosum 1923	+++	+++	+++	+++	+++	+++	+++	+++	+++	++++	No deficiency observed	Stimulated
Glenosporella Loboi 1488	+++	+++	+++	+++	+++	+++	+++	+++	+++	++++	No deficiency observed	Stimulated
Glenosporopsis amazonica 1848	-	-	++++	++++	++++	++++	++++	++++	++++	++++	Thiamin	Not stimulated
Haplosporangium parvum 2606	+++	+++	+++	+++	+++	+++	+++	+++	+++	++++	No deficiency observed	Stimulated
Hemispora stellata 1125	+++	+++	+++	+++	+++	+++	+++	+++	+++	++++	No deficiency observed	Stimulated
Histoplasma capsulatum 954	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	No deficiency observed	Somewhat stimulated
Histoplasma capsulatum 1860	+++	+++	+++	+++	+++	+++	+++	+++	+++	++++	No deficiency observed	Stimulated
Histoplasma capsulatum 2357	-	-	+++	+++	+++	+++	+++	+++	+++	++++	Thiamin	Stimulated
Hormiscium dermatitidis 1800	-	-	++++	++++	++++	++++	++++	++++	++++	++++	Thiamin	Not stimulated
Hormodendrum algeriensis 2222	-	-	++++	++++	++++	++++	++++	++++	++++	++++	Thiamin	Not stimulated
Hormodendrum Langeroni 990	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	No deficiency observed	Not stimulated
Madurella americana 1182	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	No deficiency observed	Not stimulated
Madurella Ikedai 2583	+++	++++	++++	++++	++++	++++	++++	++++	++++	++++	The mixture of the vitamins seems to stimulate growth in relation with the basal medium.	Seems stimulate growth in relation to basal medium.
Madurella mycetomi 2372	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	No deficiency observed	Not stimulated
Malassezia tropica 2430	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	No deficiency observed	Not stimulated
Microsporum Audouini 1447	-	+++	+++	+++	+	+++	+++	+++	+++	++++	Nicotinic acid	Stimulated
Microsporum canis 2555	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	No deficiency observed	Somewhat stimulated
Microsporum ferrugineum 569	+	+	+	+	+	+	+	+	+	++++	No deficiency observed	Greatly stimulated
Monosporium apiospermum 1603	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	No deficiency observed	Not stimulated
Paracoccidioides brasiliensis 2594	-	-	+++	+++	+++	+++	+++	+++	+++	++++	Thiamin	Stimulated
Paracoccidioides brasiliensis 2596	-	-	+++	+++	+++	+++	+++	+++	+++	++++	Thiamin	Stimulated
Paracoccidioides brasiliensis 2597	++	++	++++	++++	++++	++++	++++	++++	++++	++++	Thiamin (Partially deficient).	Somewhat stimulated
Phialophora compactum 1991	+	+	++++	++++	++++	++++	++++	++++	++++	++++	Thiamin	Not stimulated
Phialophora Jeanselmei 2000	-	-	++++	++++	++++	++++	++++	++++	++++	++++	Thiamin	Not stimulated
Phialophora Pedrosoi 1885	-	+	++++	++++	++++	++++	++++	++++	++++	++++	Thiamin	Not stimulated
Phialophora verrucosa 1995	-	+	++++	++++	++++	++++	++++	++++	++++	++++	Thiamin	Not stimulated
Piedraia Hortai 1538	-	-	++	++	++	++	++	++	++	++++	Thiamin	Stimulated
Piedraia Hortai 2458	-	-	++	++	++	++	++	++	++	++++	Thiamin	Stimulated
Sporotrichum Schenckii 2562	-	-	+++	+++	+++	+++	+++	+++	+++	++++	Thiamin	Stimulated
Torulopsis neoformans 2561	-	-	++++	++++	++++	++++	++++	++++	++++	++++	Thiamin	Not stimulated
Trichophyton mentagrophytes 2543	+	+	++	++	++	++	+	++	++	++++	Thiamin and Inositol	Stimulated
Trichophyton rubrum 2553	+++	+++	+++	+++	+++	+++	+++	+++	+++	++++	No deficiency observed	Stimulated
Trichophyton Sabouraudi 512	++	++	++	++	++	++	++	++	++	++++	No deficiency observed	Stimulated
Trichophyton sulfureum 1421	-	-	-	-	-	-	-	-	-	++++	No deficiency observed	Greatly stimulated
Trichophyton tonsurans 612	+	+	+	+	+	+	+	+	+	++++	No deficiency observed	Greatly stimulated
Trichophyton violaceum 1254	-	-	-	-	-	-	-	-	-	++++	No deficiency observed	Greatly stimulated
Trichosporon Beigelii 2041	-	-	++++	++++	++++	++++	++++	-	++++	++++	Thiamin and Biotin	Not stimulated
Trichosporon minor 984	-	-	++++	++++	++++	++++	++++	++++	++++	++++	Thiamin	Not stimulated

- = Absent or very slight growth.

+ to ++++ = Increasing intensity of growth.

Verifica-se pois que dentre as amostras que mostraram deficiencias, amaior percentagem corresponde à tiamina. Não foram observadas deficiencias para riboflavina, piridoxina e pantotenato de calcio.

Como fatos de maior interesse a serem assinalados, temos em primeiro lugar a verificação da exigencia de biotina por *Allescheria Boydii* (amostras nos. 386 e 1699). Até então não havia sido constatada a exigencia dessa vitamina por nenhum cogumelo patogenico filamentoso (NICKERSON & WILLIAMS, 1947). SCHOPFER & BLUMER (1942, 1943) haviam observado que *Trichophyton album* exigia biotina em determinadas condições, dependendo da idade da cultura e da composição do meio (fonte de nitrogenio) e concluem admitindo que nesse caso a biotina age como fator de crescimento complementar (SCHOPFER & BLUMER, 1943).

Quando se substitui a asparagina por sulfato de amonio ou citrato de amonio, obtem-se o mesmo resultado, isto é, o fungo não se desenvolve sem a adição de biotina ao meio de cultura. Por outro lado, o desenvolvimento do cogumelo é, dentro de certos limites, proporcional à concentração de biotina e mostra uma grande sensibilidade a essa vitamina, o que sugere a possibilidade do uso desse microorganismo na sua determinação quantitativa (CURY, 1949).

Nas amostras de *A. Boydii* estudadas (certamente essas amostras são identicas, isto é, trata-se da amostra original de Shear) não observamos a formação de peritecios. Só verificamos frutificações dos tipos *Cephalosporium* e *Dendrostilbella* (SHEAR, 1922), o que concorda com as observações de EMMONS (1944). Este autor afirma ainda no mesmo trabalho que *A. Boydii* é a forma ascocarpica de *Monosporium apiospermum*. Verificamos no presente trabalho que a amostra de *M. apiospermum* estudada (no. 1603) é autotrofica com relação às vitaminas ensaiadas. A transformação de uma forma noutra (*M. apiospermum* — *A. Boydii*), seria acompanhada por uma perda da capacidade de sintese da biotina? Só estudos mais detalhados e com maior numero de amostras poderão esclarecer este ponto.

Outro fato interessante por nós observado, se refere à exigencia de acido nicotinico pela amostra de *Microsporum Audouini* no. 1447. É grande o numero de bacterias que exige essa vitamina e nas quais ela se porta como um fator de crescimento essencial (ver a este proposito as revisões de PETERSON, 1941; LWOFF, 1943; PETERSON & PETERSON, 1945; KNIGHT, 1945; PORTER, 1946), sendo em alguns casos utilizadas na sua determinação quantitativa. Tal exigencia tambem foi observada e estudada entre leveduras fermentadoras da lactose (ROGOSA, 1943, 1944) e em especial com *Torula cremoris* (KOSER, WRIGHT & DORFMAN, 1942; KOSER & WRIGHT, 1943).

Na literatura por nós consultada, não encontramos referencias à exigencia de acido nicotinico por cogumelos filamentosos, patogenicos ou não, exceção feita das mutantes de *Neurospora crassa* (BEADLE & TATUM, 1945). Todavia, deve ser resalvado que neste caso a exigencia é artificialmente induzida, tendo se originado por mutações consequentes á ação de raios-X e de radiações ultra-violeta. Em trabalho recente CANTINO (1948) refere e estuda em detalhe a exigencia de nicotinamida por *Blastocladia Pringsheimii*, um ficomiceto aquatico da ordem *Blastocladiales*, de constituição rudimentar. No genero *Blastocladia* o talo é formado por uma celula basal simples, lobada ou ramificada, não se tratando em rigor de cogumelo filamentoso tipico.

BENEDEK (1943) estudando varias amostras de *Microsporum Audouini*, confirma as observações de CONANT (1936) de que essa especie não se desenvolve em meio de arroz polido, ao contrario das outras especies de *Microsporum* de origem animal, afirmando tratar-se de um meio seguro para a distinção das mesmas. Verificou ainda BENEDEK que em meio de arroz polido, uma das amostras estudadas teve o seu desenvolvimento grandemente estimulado pela contaminação por um bacilo que descreveu como especie nova, designando-o *Bacillus weidmaniensis*, sem apurar contudo qual a substancia ou substancias responsaveis por essa ação estimulante. HAZEN (1947) estudando o efeito do extrato de levedura, tiamina, piridoxina e do *B. weidmaniensis* sobre as caracteristicas coloniais e formação de fusos por *M. Audouini*, quando adicionados a meio basico contendo peptona e mel de abelhas, conclue que o extrato de levedura exerce um acentuado aumento não só do crescimento mas tambem da produção de macroconidios, comparavel à ação do *B. weidmaniensis*. Adicionando piridoxina ao meio basico, o crescimento difere pouco ou quasi nada daquele que se observa neste meio, mas a produção de macroconidios é mais abundante. A adição de tiamina ou de tiamina associada à piridoxina não resultou em aumento apreciavel quer do desenvolvimento da cultura, quer da produção de fusos.

Ainda, segundo os resultados obtidos, observa-se que dentro da mesma especie, diefrentes amostras mostram em certos casos, comportamento tambem diverso. É o que se verifica com *Histoplasma capsulatum*. De tres amostras estudadas, duas se mostraram autotroficas para as vitaminas ensaiadas (nos. 954 e 1860), enquanto que a terceira (no. 2357) é deficiente para tiamina.[1]) Não sabemos se este fato está ligado à idade da cultura, isto é, à condição da manutenção por longos periodos em cultivos artificiais, o que poderia acarretar modificações na sua capacidade de sintese, ou se reside simplesmente no comportamento diverso de diferentes amostras da mesma especie dada à sua variabilidade natural. Este assunto merece ser estudado com maiores detalhes.

Em relação às culturas de *Paracoccidioides brasiliensis*, verifica-se que todas as tres amostras mostraram deficiencias para a tiamina, sendo que duas delas (nos. 2594 e 2596) são totalmente deficientes para essa vitamina, enquanto que a terceira (no. 2597) mostra uma deficiencia parcial somente.

Quanto a *Coccidioides immitis*, ROESSLER *et al* (1946), verificaram que suas exigencias nutritivas eram muito simples, desenvolvendo-se bem em meio sintetico, sem exigencias vitaminicas. Nossos resultados concordam com os desses autores. Muitos outros autores são unanimes em afirmar o bom desenvolvimento do cogumelo em meios sinteticos relativamente simples (HIRSCH & BENSON, 1927; HIRSCH & D'ANDREA, 1927; STEWART & MEYER, 1938; BAKER & SMITH, 1942; HASSID, BAKER & MCCREADY, 1943). Todas as tres amostras que estudamos, na realidade se desenvolvem em meio basico, mas a intensidade do seu crescimento nesse meio é bastante reduzida em comparação com o que se observa em meio contendo extrato de levedura, que exerce uma nitida ação estimulante.

A amostra de *Achorion gypseum* (*Microsporum gypseum*) que estudamos mostrou comportamento semelhante ao que foi observado por WILLIAMS & HOHN (1932). Estes autores verificaram que *M. fulvum* (*M. gypseum*) se desenvolvia bem em uma solução mineral contendo sacarose, nitrato de potassio e fosfato de amonio. A adição de uma mistura de amino acidos melhorou o crescimento, mas o extrato de levedura mostrou-se mais ativo.

Com relação a *Achorion Schoenleinii* (*Trichophyton Schoenleinii*) a amostra que estudamos não mostrou exigencias para as vitaminas ensaiadas. Na literatura consultada, não encontramos referencia a estudos sobre exigencias vitaminicas desta especie. BURKHOLDER & MOYER (1943) verificaram que *Trichophyton faviforme* era deficiente para tiamina e inositol. Não sabemos porem a que especie esses autores se referem. O que ha na realidade é o grupo faviforme. SABOURAUD (1910) denomina ,,Trichophytons faviformes" e inclui no grupo dos tricofitos ectotrix megasporados as tres especies: *T. album, T. discoides* e *T. ochraceum*. CONANT *et al* (1944) admitem que *T. discoides, T. ochraceum* e *T. album* sejam sinonimos de *T. Schoenleinii*. NICKERSON & WILLIAMS (1947) tambem são dessa opinião, uma vez que transcrevendo os resultados obtidos por SCHOPFER & BLUMER (1942) com *T. album* e os de ROBBINS, MACKINNON & MA (1942) com *T. discoides*, dão estas duas especies como sinonimas de *T. Schoenleinii*. MACKINNON & A-ALLENDE (1948) não concordam com essa sinonimia por varias e justas razões, se bem que considerem as especies *T. album, T. discoides* e *T. ochraceum* como sinonimas, opinião da qual compartilhamos. Nesse proposito, o magnifico trabalho de SABOURAUD (1910) é muito claro e não deixa a menor duvida quanto às diferenças existentes entre *T. (A.) Shcoenleinii* de um lado e *T. discoides, T. ochraceum* e *T. album* do outro.

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[1]) Esta amostra foi isolada de cão por PARA (1946). Segundo esse autor, o cogumelo se desenvolvia bem em meio de Czapek. O nosso resultado difere pois do que foi observado, uma vez que essa amostra se mostrou deficiente para tiamina.

SCHOPFER & BLUMER (1942, 1943) estudando as exigencias vitaminicas de *T. album* verificaram que as mesmas eram condicionadas pela idade da cultura e pela composição do meio (fonte de nitrogenio). Usando asparagina como fonte de nitrogenio, só a tiamina era necessaria. O cogumelo, nessas condições só exigia biotina no inicio do crescimento, pois, decorridos alguns dias, o crescimento dos testemunhas era identico ao das culturas supridas com biotina. Modificando a composição do meio, para que se obtivesse um crescimento maximo, era necessaria a adição de tiamina, biotina, *i*-inositol e piridoxina. Concluem esses autores admitindo no caso de *T. album* a biotina se comporta como fator de crescimento complementar (SCHOPFER & BLUMER, 1943).

MACKINNON (1942) observou que culturas de *Actinomyces albus* tinham efeito estimulante sobre o crescimento de *T. discoides* em meio de glucose-peptona-agar e que a adição de tiamina exercia o mesmo efeito. ROBBINS, MACKINNON & MA (1942) estudando em detalhe o comportamento da amostra anteriormente utilizada por MACKINNON, verificaram que a mesma era completamente deficiente para tiamina molecular, *dl*-inositol, e piridoxina. Alem dessas deficienicas que eram totais, havia tambem deficiencias parciais para substancias não identificadas presentes na peptona, hidrolisado de caseina, hidrolisado de ovo-albumina, extrato de malte, gelatina e um filtrado preparado de batatas, (fração Dr.). Essas deficiencias não puderam ser satisfeitas por biotina, riboflavina, acido folico, acido pimelico, acido p-aminobenzoico, acido pantotenico, hipoxantina, guanina, nicotinamida, diacetato de 2-metil-1,4-naftoquinona, sete bases puricas e pirimidicas e 43 amino acidos. MACKINNON & A-ALLENDE (1948) estudando as exigencias vitaminicas de 5 amostras de *T. discoides*, verificaram que tres amostras eram completamente deficientes para dl-inositol, uma para tiamina e outra para dl-inositol, tiamina e piridoxina.

OYAMA (1937) observou que *Grubyella ochracea* (*T. ochraceum*) se desenvolvia mal em meio contendo cloreto de sodio, lactato de amonio, fosfato de sodio, asparagina e um açucar. A adição de tiamina era sem efeito, mas um concentrado de polimento de arroz permitia bom crescimento. MACKINNON & A-ALLENDE (1948) em duas amostras de *T. ochraceum* estudadas, verificaram que uma delas era completamente deficiente para dl-inostitol e a outra para dl-inositol e tiamina.

A amostra de *T. Schoenleinii* por nós estudada, não mostrou exigencia para as vitaminas ensaiadas, sendo que o extrato de levedura tem uma intensa ação favorecedora do crescimento.

Actinomyces madurae apresentou bom desenvolvimento em meio basico se bem que o extrato de levedura tenha mostrado uma ação nitidamente favorecedora do crescimento. HERRICK & ALEXOPOULUS (1943) já haviam observado que essa especie se desenvolvia bem em meio mineral basico contendo asparagina e saracose e que nestas condições sintetisava quantidades de tiamina suficientes para suportar bom crescimento de *Phycomyces Blakesleeanus*.

Os resultados que obtivemos com duas amostras de *Blastomyces dermatitidis* concordam com o que foi verificado por STEWART & MEYER (1938), isto é, o cogumelo não exige vitaminas para o seu crescimento. Esses autores obtiveram boas culturas em meio sintetico com fontes de carbono e de nitrogenio relativamente simples.

Em *Candida suaveolens*, BURKHOLDER (1943) observou que a amostra Y-699 era deficiente para tiamina e biotina. Em trabalho posterior, BURKHOLDER, MACVEIGH & MOYER (1944), relatam que a amostra estudada exigia somente biotina. A amostra que estudamos mostrou deficiencia somente para tiamina, diferindo pois, neste particular, das duas outras amostras referidas. Trata-se como já tivemos oportunidade de relatar, da amostra descrita por MAGALHÃES (1932) como *Neogeotrichum pulmoneum*.

Com relação a *Cephalosporium recifei*, ARÊA LEÃO & LOBO (1934) ao descrever o cogumelo como especie nova, relatam o seu desenvolvimento em meio de Czapek. Tambem ALMEIDA & SIMÕES BARBOSA (1940) confirmam esse fato, admitindo que nesse meio o cogumelo tem um aspeto caracteristico. A amostra que utilizamos no presente trabalho é a amostra original descrita por ARÊA LEÃO & LOBO e mostrou comportamento semelhante ao que foi referido por esses autores.

Os resultados que obtivemos com *Debaryomyces Hudeloi* concordam com os de BURKHOLDER, MACVEIGH & MOYER (1944), que verificaram ser essa levedura deficiente para biotina.

Do mesmo modo que PECK & ROSENFELD (1938) e ROBBINS & MACVEIGH (1946), não observamos exigencias vitaminicas com *Epidermophyton floccosum*, que se desenvolveu bem em todos os meios. Tambem obtivemos os mesmos resultados que estes ultimos autores com relação a *Microsporum canis*, que não mostrou exigencia para qualquer das vitaminas utilizadas.

Com *Phialophora Pedrosoi* e *P. verrucosa* tambem são concordantes os nossos resultados com o que verificaram BURKHOLDER & MOYER (1943), pois ambas são deficientes para tiamina, o mesmo se dando com *Sporotrichum Schenckii*, com a diferença porem que na amostra por nós estudada o extrato de levedura exerce uma clara ação estimulante, enquanto que aqueles autores referem que a junção de peptona ou de concentrado de figado não exercia ação estimulante alguma.

Quanto à amostra de *Torulopsis neoformans* por nós estudada, obtivemos comportamento semelhante ao que é referido por OYAMA (1937) de que a adição de tiamina ao meio basico mineral melhorava o crescimento. No caso presente, a adição de tiamina traz em consequencia um crescimento abundante em contraste com o que se observa no meio basico e no meio em que falta essa vitamina, onde o seu desenvolvimento é praticamente nulo. Tambem ASCHNER, MAGER & LEIBOWITZ (1945) e MAGER & ASCHNER (1946, 1947), estudando a produção extracelular de amido por leveduras capsuladas, observaram que *T. neoformans* se desenvolvia bem em meio mineral simples, com sulfato de amonio como fonte de nitrogenio, ao qual adicionavam unicamente tiamina.

MOSHER *et al* (1936) observaram que *Trichophyton interdigitale* (*T. mentagrophytes*) não se desenvolvia em meio basico mineral contendo amino acidos.

A adição de tiamina, inositol e de uma preparação altamente concentrada de acido pantotenico ou de lactoflavina bruta permitia o crescimento. Combinações dessas vitaminas eram mais ativas que o seu uso isolado. PECK & ROSENFELD (1938) verificaram que a adição das vitaminas A, B e D não influia sobre o crescimento de *T. gypseum* (*T. mentagrophytes*) e que a vitamina C tinha nitido efeito fungistatico e fungicida. BURKHOLDER & MOYER (1943) estudando 5 amostras de *T. mentagrophytes* não observaram deficiencias vitaminicas para as mesmas e só uma delas tinha o seu crescimento estimulado pela adição de figado ou de peptona. ROBBINS & MA (1945) em excelente trabalho no qual estudam minuciosamente as exigencias em fatores de crescimento de *T. mentagrophytes*, concluem que a amostra estudada é autotrofica para as vitaminas ensaiadas, observando efeito benefico com a adição de peptona e de hidrolisado de caseina ou de outras proteinas. Atribeum essa ação estimulante aos amino acidos presentes nesses hidrolisados. ROBBINS & MCVEIGH (1946) confirmam a auxo-autotrofia desse dermatofito. A amostra que estudamos mostrou-se deficiente para tiamina e inositol. Neste particular, nossa amostra se aproxima daquela estudada por MOSHER *et al* (1936).

Quanto a *Trichophyton rubrum*, BURKHOLDER & MOYER (1943) verificaram que esse dermatofito não apresentava exigencias vitaminicas e que a adição de concentrado de figado ou peptona tinha uma pequena ação estimulante. ROBBINS & MCVEIGH (1946) tambem observaram bom desenvolvimento desse fungo em meio basico mineral contendo glucose e asparagina. Melhor crescimento foi observado pela adição de hidrolisado de caseina. BENHAM (1948) observou que de 50 amostras de *T. rubrum* cultivadas em "blood agar base" (Difco) 49 mostravam a formação dos fusos tipicos. Admite que a substancia responsavel pela formação desse tipo de esporos seja a triptose, uma vez que a sua supressão do meio traz como consequencia a não formação dos macroconidios.

BURKHOLDER & MOYER (1943) relatam que a amostra de *Trichophyton acuminatum* (*T. Sabouraudi*) por eles estudada era parcialmente deficiente para tiamina e que a junção de peptona ou de concentrado de figado não tinha ação estimulante. Fato semelhante, esses mesmos autores observaram com *Trichophyton sulfureum*. A amostra de *T. Sabouraudi* que estudamos, não mostrou exigencia para qualquer das vitaminas usadas mas o extrato de levedura mostrou uma nitida ação estimulante do crescimento. Quanto a *T. sulfureum*, nossa amostra mostrou comportamento absolutamente diverso do que foi observado pelos autores acima referidos. O seu desenvolvimento em todos os meios, com exceção daquele contendo extrato de levedura, foi nulo, praticamente reduzido ao inoculum. Apesar de não se mostrar exigente para qualquer das vitaminas ensaiadas, o extrato de levedura mostrou uma intensa ação favorecedora do crescimento.

OYAMA (1937) relata que *Trichophyton crateriforme* (*T. tonsurans*) cresce lentamente em meio mineral e que a adição de tiamina não tinha efeito estimulante algum. Contudo, a adição de um concentratrado de polimento de arroz, tinha ação favorecedora do crescimento. Nossos resultados se aproximam dos desse autor, uma vez que a amostra que estudamos não se mostrou exigente para qualquer das vitaminas estudadas, sendo que o extrato de levedura mostrou uma intensa ação favorecedora do crescimento.

Em *Trichophyton violaceum*, BURKHOLDER & MOYER (1943) verificaram deficiencia para tiamina e grande estimulo do crescimento pela adição de peptona ou de concentrado de figado. A amostra por nós estudada não mostrou deficiencia para qualquer das vitaminas ensaiadas. O seu desenvolvimento em todos os meios, com exceção daquele contendo extrato de levedura, foi nulo, reduzido ao inoculum, sendo que o extrato de levedura mostrou intensa ação favorecedora do crescimento.

Quanto a *Trichosporon Beigelii*, OYAMA (1937) já havia verificado que esse cogumelo se desenvolvia mal em meio mineral. A adição de tiamina em nada melhorava o crescimento, mas a adição de um concentrado de polimento de arroz mostrava boa ação estimulante. A amostra que estudamos exige tiamina e biotina, não se desenvolvendo nos meios em que qualquer uma dessas vitaminas seja excluida.

Com relação a *Trichosporon minor*, ARÊA LEÃO (1940) ao descrever esse cogumelo como especie nova, já havia observado que o mesmo se desenvolvia mal e muito lentamente em meio de Czapek-Dox, e que o crescimento se fazia quasi exclusivamente na profundidade do meio de cultura. A amostra que utilizamos no presente trabalho é a amostra original descrita pelo autor acima referido, não se desenvolvendo em meio basico nem no meio em que a tiamina foi excluida.

Em nossa opinião, o estudo da nutrição e do metabolismo dos cogumelos patogenicos apresenta grande interesse, não só por permitir um melhor conhecimento da biologia desses microorganismos, mas tambem porque pensamos ser de utilidade o estabelecimento e o emprego de meios de cultura de composição quimica definida, nos quais se possa cultivar cogumelos dos mais diversos tipos nutritivos e das mais variadas exigencias vitaminicas. Uma vez conseguidos esses meios, o estudo da morfologia desses microorganismos tornar-se-ia mais uniforme, pois os mesmos meios poderiam ser usados por todos os estudiosos do assunto, dada à constancia da sua composição. Por outro lado, o melhor conhecimento da nutrição desses seres apresenta ainda a possibilidade da utilização dos dados adquiridos, na sistematica desse grupo, assaz dificil e ainda um tanto nebulosa. Nesse sentido varios são os trabalhos realizados, podendo-se citar dentre outros o de WICKERHAM (1946) e WICKERHAM & BURTON (1948) sobre a assimilação de compostos carbonados e nitrogenados por leveduras ,o de SCHULTZ, ATKIN & FREY (1940) tentando estabelecer uma classificação bioquimica para certo grupo de leveduras e o de SCHOPFER (1944), sobre o genero *Candida*.

Todavia, é necessaria cautela na interpretação dos resultados e na sua aplicação, como bem ponderam SCHOPFER & GUILLOUD (1945), afim de não se chegar a exageros ou a cometer erros, aliás evitaveis. O trabalho de MACKINNON & A-ALLENDE (1948) é bem sugestivo neste sentido. Esses autores, estudando as exigencias vitaminicas de *Trichophyton discoides* e de *Trichophyton ochraceum*, verificaram que em 5 amostras da primeira especie, tres eram os tipos de exigencias vitaminicas observadas, enquanto que duas amostras da segunda especie tambem mostravam comportamento diverso. E, se se considera essas duas especies como sinonimas (CONANT *et al*, 1944; MACKINNON & A-ALLENDE, 1948), chega-se à conclusão de que em 7 amostras estudadas, 4 são os tipos de exigencias vitaminicas apresentadas. Naturalmente seria absurdo, só por esse fato, admitir-se que se tratam de 4 especies diferentes.

Evidentemente, os resultados por nós obtidos só serão considerados validos para as amostras estudadas e nas condições da nossa experimentação, não se podendo aplica-los às diversas especies, dada a sua grande variabilidade não só morfologica mas tambem fisiologica.

Para o futuro, nossas pesquisas serão conduzidas da modo a estudarmos mais minuciosamente a nutrição e as exigencias vitaminicas de cada grupo, genero, especie ou amostra. Os diversos grupos de cogumelos patogenicos devem ser melhor estudados neste particular. Com os conhecimentos adquiridos, muitos dos problemas até então obscuros, relacionados ao modo de infecção, patogenicidade e ao habitat natural desses microorganismos, talvez possam ser esclarecidos (NICKERSON, 1948).

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Sumario

As exigencias vitaminicas de 60 amostras de cogumelos patogenicos, pertencentes a 50 especies e a 31 generos foram estudadas, utilizando-se meio basico mineral contendo glucose e asparagina, suplementado com 7 diferentes vitaminas. Tambem foi empregado meio basico adicionado de extrato de levedura, servindo para comparação com os demais, bem como para a verificação da sua ação estimulante sobre o crescimento das amostras utilizadas. Das 60 amostras estudadas, 35 se mostraram autotroficas e as restantes, heterotroficas para as vitaminas ensaiadas. Destas, as amostras deficientes para tiamina representam a maior percentagem. Alem das deficiencias simples, isto é, para uma só vitamina, observou-se tambem em algumas amostras, deficiencias para mais de uma vitamina. Não foram verificadas deficiencias para riboflavina, piridoxina e pantotenato de calcio.

Como fatos mais interessantes a ser assinalados, contam-se a verificação da exigencia de biotina por *Allescheria Boydii* e a deficiencia de acido nicotinico na amostra de *Microsporum Audouini* estudada.

E feita a revisão bibliografica diretamente relacionada às especies estudadas e devidamente encarecida a importancia que o estudo da nutrição e das exigencias vitaminicas dos cogumelos patogenicos possa ter para o melhor conhecimento da biologia desses microorganismos.

Vitamin deficiencies of pathogenic fungi

Summary, Results, and Discussion

The vitamin requirements of pathogenic fungi have not been studied with sufficient attention, relatively few papers having been published on the subject. The present paper deals with the vitamin requirements of 60 strains of pathogenic fungi belonging to 50 species and 31 genera. The basal medium used had the following composition per liter: dextrose 30 g, asparagin (recrystallized) 2 g, KH_2PO_4 1.5 g, $MgSO_4.7H_2O$ 0.5 g, agar (purified) 12 g, and trace elements (B, Cu, Fe, Mn, Mo, and Zn). The vitamins used were the following: thiamin (hydrochloride), riboflavin, pyridoxin (hydrochloride), nicotinic acid, calcium pantothenate, inositol, and biotin. Details on the composition of the medium, strains used, glassware, inocula, estimation of growth, are given in the text.

Of the 60 strains studied, 35 did not require any of the vitamins tested (auxo-autotrophic): *Achorion gallinae, A. gypseum, A. Schoenleinii, Acremoniella Lutzi, Actinomyces brasiliensis, A. madurae, Blastomyces dermatitidis* (2 strains), *Cephalosporium recifei, Cladosporium Wernecki* 1886 C and 1886 M, *Coccidioides immitis* (3 strains), *Endodermophyton concentricum, E. Roquettei, E. tropicale, Epidermophyton floccosum, Glenosporella Loboi, Haplosporangium parvum, Hemispora stellata, Histoplasma capsulatum* (2 strains), *Hormoden-*

drum Langeroni, Madurella americana, M. mycetomi, Malassezia tropica, Microsporum canis, M. ferrugineum, Monosporium apiospermum, Trichophyton rubrum, T. Sabouraudi, T. sulfureum, T. tonsurans, and *T. violaceum.*

The 25 strains requiring one or more of the vitamins, can be thus distinguished:

a) The mixture of the vitamins seems to stimulate growth in comparison with the basal medium — 1 strain (*Madurella Ikedai*).

b) Completely deficient for thiamin — 17 strains (*Acremonium Potronii, Candida suaveolens, Glenosporopsis amazonica, Histoplasma capsulatum* no. 2357, *Hormiscium dermatitidis, Hormodendrum algeriensis, Paracoccidioides brasiliensis* 2594, *P. brasiliensis* 2596, *Phialophora compactum, P. Jeanselmei, P. Pedrosoi, P. verrucosa, Piedraia Hortai* 1538, *P. Hortai* 2458, *Sporotrichum Schenckii, Torulopsis neoformans,* and *Trichosporon minor.*

c) Partially deficient for thiamin — 1 strain (*Paracoccidioides brasiliensis* 2597).

d) Deficient for biotin — 3 strains (*Allescheria Boydii* 386, *A. Boydii* 1699, and *Debaryomyces Hudeloi*).

e) Deficient for nicotinic acid -- 1 strain (*Microsporum Audouini*).

f) Deficient for thiamin and biotin — 1 strain (*Trichosporon Beigelii*).

g) Deficient for thiamin and inositol — 1 strain (*Trichophyton mentagrophytes*).

Thus, it was found that among the deficient strains, the majority required thiamin. No strains were found to require riboflavin, pyridoxin or calcium pantothenate.

Worthy of notice is the finding that *Allescheria Boydii* required biotin for growth (figs. 8 and 9). Up to the present time no pathogenic filamentous fungi have been shown to require this vitamin* (NICKERSON & WILLIAMS, 1947). SCHOPFER & BLUMER (1942, 1943) showed that *Trichophyton album* required biotin under certain conditions, depending on the age of the culture and composition of the medium (nitrogen source), concluding that in this case biotin acted as a complementary growth factor (SCHOPFER & BLUMER, 1943).

The changing of asparagin in the culture medium by ammonium sulfate or ammonium citrate does not alter the results, the fungus still requiring biotin for growth. Furthermore, this growth is within limits proportional to biotin concentration, being very sensitive to this vitamin, which suggests, the possibility of the use of this microorganism in the assay of biotin (CURY 1949).

The strains of *A. Boydii* studied (which are surely identical being from the original strain of SHEAR) did not show perithecium formation. The only types of frutification observed were of *Cephalosporium* and *Dendrostilbella* (SHEAR, 1922), which agrees with the observation of EMMONS (1944). This author states that *A. Boydii* is the ascocarpic form of *Monosporium apiospermum.* The strain of *M. apiospermum* (1603) studied in the present paper is autotrophic in relation to the vitamins tested. The transformation of *M. apiospermum* to *A. Boydii* would be acompanied by a loss of the capacity to synthesize biotin? This point can only be cleared by further study of varied strains.

Another interesting result from our study is the fact that *Microsporum Audouini* no. 1447 required nicotinic acid for growth. This vitamin is an essential growth factor for a number of bacteria (see reviews by PETERSON, 1941; LWOFF, 1943; PETERSON & PETERSON, 1945; KNIGHT, 1945; PORTER,

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* This paper was in press, when SALVIN (J. Inf. Dis., 84: 275-283, 1949) reported that biotin was necessary for the development of the yeastlike-phase of *Histoplasma Capsolatum* and that only one strain of six strains tested required biotin for the mycelial growth.

1946), some being used for its quantitative determination. This requirement was also observed among lactose-fermenting yeasts (ROGOSA, 1943, 1944), specially with *Torula cremoris* (KOSER, WRIGHT & DORFMAN, 1942; KOSER & WRIGHT, 1943).

We have not found in the literature references to pathogenic or non-pathogenic filamentous fungi requiring nicotinic acid for growth, with the exception of mutants of *Neurospora crassa* (BEADLE & TATUM, 1945); it must be stressed however, that in this case this character was artificially induced by X-rays and ultra-violet radiations. In a recent work, CANTINO (1948) showed the requirement of nicotinamide by *Blastocladia Pringsheimii*, an aquatic Phycomycete of very rudimentary structure belonging to the order *Blastocladiales*. In the genus *Blastocladia* the thallus is formed by a simple, lobate or branched basal cell. Besides nicotinamide, this Phycomycete is shown to require thiamin and biotin.

BENEDEK (1943), studying several strains of *M. Audouini* confirmed the finding of CONANT (1936) that this species does not grow in polished rice medium, as opposed to other species of *Microsporum* from animal origin, suggesting this character as a reliable test for distinguishing these species. BENEDEK further found that in polished rice medium one of the strains of *M. Audouini* had its growth markedly stimulated following the contamination by a new species of bacillus which he named *Bacillus weidmaniensis*; the growth stimulating factor was not identified. HAZEN (1947) studying the action of yeast extract, thiamin, pyridoxin, and of *B. weidmaniensis* on the colonial characteristics and macroconidia formation by *M. Audouini*, using a basal medium containing peptone and honey, concluded that yeast extract markedly stimulated not only growth but also macroconidia formation, which is comparable with the action of *B. weidmaniensis*. Pyridoxin has practically no effect on growth but stimulates macroconidia formation. Thiamin alone or associated with pyridoxin did not stimulate growth nor macroconidia formation. Our strain showed practically no growth in medium 1 and slight growth in medium 5. It grew well in all other media specially in the one containing yeast extract which has a stimulating action (fig. 37). It is easily shown to require nicotinic acid.

Our results further show differences in the behaviour of strains within the same species, such as in the case of *Histoplasma capsulatum*; of the three strains studied, two are autotrophic for the vitamins tested (nos. 954 and 1860) (figs. 27 and 28) while the third (no. 2357) requires thiamin (fig. 29). This is the strain isolated by PARA (1946) from a dog, and according to this author, it grew well in Czapek medium. Our results differ from those of this author, since the same strain showed to be completely deficient for thiamin.

It is not known whether this difference depends on the age of the culture being maintained in artificial culture media which might bring about alterations in its synthesizing capacities or simply in differences due to natural variation. This subject deserves more detailed studies.

In the case of *Paracoccidioides brasiliensis*, all the three strains studied required thiamin, two of them (nos. 2594 and 2596) not growing at all in the absence of this vitamin (fig. 41) while the third one (no 2597) showed partial deficiency (fig. 42).

Our results with *Coccicioides immitis* agree with those of ROESSLER *et al* (1946) who showed that this fungus has very simple nutritional requirements growing well in synthetic media without vitamins. Several other authors are unanimous in finding it to grow well in relatively simple synthetic media (HIRSCH & BENSON, 1927; HIRSCH & D'ANDREA 1927; STEWART & MEYER, 1938; Baker & Smith, 1942; Hassid, Baker & McCready, 1943). Our three strains grew in the basal medium, but their growth was markedly improved by yeast extract which has an obvious stimulating action (figs. 15, 16 and 17).

Achorion gallinae 1456 showed good growth in all media, more abundant however in the medium containing yeast extract which has a stimulating action. None of the vitamine tested were required for growth (fig. 1).

The strain of *Achorion gypseum* (*Microsporum gypseum*) 1439 behaved in agreement with the results obtained by Williams & Hohn (1932). These authors showed that *M. fulvum* (*M. gypseum*) grew well in a synthetic medium containing sucrose, sodium nitrate and ammonium phosphate; growth was improved by the addition of a mixture of amino acids, but yeast extract had an even more active growth promoting action. Our strain showed good growth in all media but yeast extract had a stimulating action. (fig. 2).

The strain of *Achorion Schoenleinii* (*Trichophyton Schoenleinii*) did not require for growth any of the vitamins tested. We have not found in the literature any reference to the vitamin requirements of this species. Burkholder & Moyer (1943) found that *Trichophyton faviforme* required thiamin and inositol. The species referred to by these authors is uncertain; what there is in reality is the faviform group. Sabouraud (1910) named "Trichophytons faviformes" and included in the group of the ectothrix, large-spored Trichophytons the three species: *T. album, T. discoides, and T. ochraceum.* Conant *et al* (1944) admit *T. discoides, T. ochraceum* and *T. album* as synonyms of *T. Schoenleinii.* Nickerson & Williams (1947) referring to the results obtained by Schopfer & Blumer (1942) with *T. album* and those of Robbins, Mackinnon & Ma (1942) with *T. discoides,* are of the same opinion. Mackinnon & A.-Allende (1948) give several good reasons for not agreeing with this view, considering however as synonyms the species *T. album, T. discoides* and *T. ochraceum,* an opinion with which we agree. In this connection, Sabouraud (1910) is very clear in not leaving the slightest doubt as to the differences between on one side, *A. Schoenleinii,* and on the other, *T. discoides, T. album* and *T. ochraceum.*

Schopfer & Blumer (1942, 1943) found that the vitamin requirements of *T. album* were dependent on the age of the culture and composition of the medium (nitrogen source). Using asparagin as nitrogen source, only thiamin was required. The fungus only required biotin at the initial stages of growth, for, after a few days the growth of the controls was comparable with that of the cultures supplied with biotin. In order to obtain maximal growth it was necessary to add thiamin, biotin, *i*-inositol, and pyridoxin. The authors concluded that biotin is a complementary growth factor for *T. album* (Schopfer & Blumer, 1943).

Mackinnon (1942) found that cultures of *Actinomyces albus* had a growth stimulating effect on *T. discoides,* on glucose-peptone agar, and that thiamin had the same action. Robbins, Mackinnon & Ma (1942), studying this same strain in detail found it required pyridoxin, *i*-inositol and molecular thiamin. Besides being completely deficient for these vitamins the strain studied was partially deficient for unidentified substances present in peptone, casein hydrolysate, hydrolyzed egg albumin, malt extract, gelatin and a filtrate obtained from white potatoes (Dr fraction). These partial deficiencies could not be satisfied by biotin, lactoflavin, folic acid, pimelic acid, p-aminobenzoic acid, pantothenic acid, hypoxanthine, guanine, nicotinamide, 2-methyl-1,4-naphtoquinone diacetate, seven pyrimidine and purine bases, and 43 amino-acids. A phosphatide and calcium phytate containing inositol were less effective than *i*-inositol. Mackinnon & A.-Allende (1948) studying 5 strains of *T.*

discoides, found 3 requiring *dl*-inositol, one requiring thiamin, and one requiring *dl*-inositol, thiamin, and pyridoxin.

OYAMA (1937) obtained poor growth of *Grubyella ochracea* (*T. ochraceum*) in a medium containing sodium chloride, ammonium lactate, sodium phosphate, asparagine and a sugar. The addition of thiamin was without effect but a concentrate of rice polishings enhanced growth. MACKINNON & A.-ALLENDE (1948) studying two strains of *T. ochraceum* found that one required *dl*-inositol for growth and the other required *dl*-inositol and thiamin.

We emphasize that we have not found in the literature references to the vitamin requirements of *Trichophyton* (*Achorion*) *Schoenleinii*. Our strain showed no growth in the basal medium. It grew poorly in media 2 to 9 and abundantly in the medium containing yeast extract which has a striking growth promoting action. This strain did not require the vitamins tested for growth (fig. 3).

Acremoniella Lutzi 866 showed good growth in all media. It did not require for growth, the vitamins tested; yeast extract had no apparent stimulating action (fig. 4).

Acremonium Potronii 1610 showed no growth in media 1 and 2, but good growth in the remaining ones. It produced a brown pigment that diffused through the medium. Yeast extract had no apparent growth promoting action. It required thiamin for growth (fig. 5).

Actinomyces brasiliensis 2590 showed good growth in all media. Yeast extract however had a stimulating action, promoting better growth. It did not require the vitamins tested (fig. 6).

Actinomyces madurae 2593 showed good growth in the basal medium, but yeast extract had a definite stimulating action (fig. 7). HERRICK & ALEXOPOULUS (1943) had shown that this species grew well in a basal mineral medium containing asparagin and sucrose, and that under these conditions it synthesized sufficient amounts of thiamin to promote good growth of *Phycomyces Blakesleeanus*.

Our results with two strains of *Blastomyces dermatitidis* (nos. 1901 and 1902) are in agreement with those of STEWART & MEYER (1938) in that this fungus does not require vitamins for growth. These authors obtained good growth in synthetic media with relatively simple carbon and nitrogen sources.

BURKHOLDER (1943) showed that the strain Y-699 of *Candida suaveolens* required thiamin and biotin for growth. In a subsequent paper BURKHOLDER, MCVEIGH & MOYER (1944), showed that the strain studied required only biotin. Our strain required only thiamin (fig. 12), differing in this particular from the two strains referred to. Our strain, as mentioned before, is the one described by MAGALHÃES (1932) as *Neogeotrichum pulmoneum*.

With *Cephalosporium recifei*, ARÊA LEÃO & LOBO (1934) describing this fungus as a new species, stated that it grew in Czapek medium. ALMEIDA & SIMÕES BARBOSA (1940) confirmed this finding, admiting that on this medium the fungus has a characteristic appearence. The strain which we used is the original strain described and it showed the same behaviour as obtained by those authors.

Cladosporium Wernecki 1886 C and 1886 M grew well in all media, do not requiring any of the vitamins tested. Yeast extract had no stimulating action (figs. 13 and 14).

Our results with *Debaryomyces Hudeloi* are in agreement with those of BURKHOLDER, MCVEIGH & MOYER (1944) who found that this yeast required biotin for growth. Our strain showed the same behaviour, its growth being stimulated by yeast extract (fig. 18).

Endodermophyton concentricum, E. Roquettei, and *E. tropicale* behaved similarly, growing well in all media, not requiring any of the vitamins tested. Yeast extract had a slight stimulating action on the growth of these species (figs. 19, 20, 21).

Our results confirm those of Peck & Rosenfeld (1938) and Robbins & McVeigh (1946) for *Epidermophyton floccosum* (strain 1923), which grew well in all media, its growth being stimulated by yeast extract (fig. 22). With *Microsporum canis* our results were similar to those of the last two authors who showed it to grow well in a basal mineral medium containing asparagin or casein hydrolysate. Our strain grew well in all media. Yeast extract had a slight growth stimulating action (fig. 38).

Glenosporella Loboi grew well in all media. Growth was slightly better in the presence of yeast extract which had a growth stimulating action (fig. 23).

Glenosporopsis amazonica did not grow on media 1 and 2. Good growth on the remaining media. It required thiamin for growth. Yeast extract had no evident stimulating action (fig. 24).

Haplosporangium parvum showed good growth on all media; yeast extract stimulated growth (fig. 25).

Hormodendrum algeriensis showed almost no growth on media 1 and 2 and good growth on the remaining ones. Yeast extract had no evident stimulating action. It required thiamin (fig. 31).

Hormodendrum Langeroni did not require any of the vitamins, growing well on all media. Yeast extract without stimulating action (fig. 32).

Hormiscium dermatitidis showed almost no growth on media 1 and 2 (submerged mycelium). Good growth on the remaining media. Yeast extract without evident growth stimulating action. It required thiamin for growth (fig. 30).

Madurella americana did not require any of the vitamins, growing well on all media. Yeast extract without stimulating action (fig. 33).

Madurella mycetomi did not require any of the vitamins, growing well in all media. Yeast extract without stimulating action. The same applies to *Malassezia tropica* (figs. 35 and 36).

Madurella Ikedai showed reduced growth on the basal medium as compared with the remaining ones. The mixture of the vitamins seems to have a growth stimulating action in relation to the basal medium (fig. 34).

Microsporum ferrugineum grew poorly on all media with the exception of the medium containing yeast extract. It did not require any of the vitamins tested. Yeast extract had a marked growth stimulating action (fig. 39).

Monosporium apiospermum showed good growth in all media. It is autotrophic for the vitamins tested. Yeast extract had no stimulating action (fig. 40).

Phialophora compactum and *Phialophora Jeanselmei* behaved similarly showing poor growth (submerged mycelium) on media 1 and 2, and abundant growth on the remaining ones. Yeast extract without evident stimulating action (figs. 43 and 44).

For *Phialophora Pedrosoi* and *P. verrucosa* our results agree with those of Burkholder & Moyer (1943), both requiring thiamin for growth (figs. 45 and 46), the same applying to *Sporotrichum Schenckii* (fig. 49), with the difference that in this case, our strain was clearly stimulated by yeast extract whereas those authors were not able to show any stimulating action of liver and peptone.

Piedraia Hortai (strains 1538 and 2458) showed poor if any growth on media 1 and 2, moderate growth on the remaining media and abundant growth in the presence of yeast extract. A dark diffusible pigment was produced on all media, particularly by strain no. 1538. Both strains required thiamin for growth and were stimulated by yeast extract (figs. 47 and 48).

Our strain of *Torulopsis neoformans* behaved in agreement with the results obtained by OYAMA (1937) who showed that the addition of thiamin or of a concentrate of rice polishings to a basal mineral medium improved growth of *Cryptococcus* (Busse-Buschke). In our case, the addition of thiamin brings about abundant growth, in contrast with the basal medium and in thiamin-free medium where there was practically no growth (fig. 50), ASCHNER, MAGER & LEIBOWITZ (1945) and MAGER & ASCHNER (1946, 1947), studying the extracellular production of starch by capsulated yeasts found that *T. neoformans* grew well in a simple mineral medium with ammonium sulfate as nitrogen source, to which only thiamin was added.

MOSHER *et al* (1936) showed that *Trichophyton interdigitale* (*T. mentagrophytes*) would not grow in a basal mineral medium containing amino acids. Addition of thiamin, inositol, and a highly concentrated preparation of pantothenic acid, or crude lactoflavin, promoted growth. Combinations of these vitamins were more active than each one by itself. PECK & ROSENFELD (1938) found that the addition of vitamins A, B, and D had no action on the growth of *T. gypseum* (*T. mentagrophytes*) and that vitamin C had a definite fungistatic and fungicidal effect. BURKHOLDER & MOYER (1943) studying 5 strains of *T. mentagrophytes* did not find any of them to require vitamins, and only one strain having its growth stimulated by liver or peptone. ROBBINS & MA (1945) in an excellent paper where the growth factor requirements of *T. mentagrophytes* are studied in detail, concluded that their strain was autotrophic for the vitamins tested, its growth being enhanced by the addition of peptone, casein hydrolyzate, or other protein hydrolyzates, this stimulating action being attributed to the amino acids present in these hydrolyzates. ROBBINS & MCVEIGH (1946) confirmed the auxo-autotrophy of this dermatophyte. Our strain required thiamin and inositol for growth, yeast extract having a growth stimulating action (fig. 51). It is in this particular, similar to that studied by MOSHER *et al* (1936).

BURKHOLDER & MOYER (1943) showed that *Trichophyton rubrum* did not require vitamins for growth, being slightly stimulated by the addition of peptone or liver concentrate. ROBBINS & MCVEIGH (1946), also showed good growth of this fungus in a basal mineral medium containing glucose and asparagin. Better growth occurred after the addition of casein hydrolyzate. BENHAM (1948) studying 50 strains of *T. rubrum* grown in blood agar base (Difco) obtained typical macroconidia formation in 49, admitting that the active substance which brings about this type of sporulation is tryptose, since in its absence, it was not observed. Our strain showed good growth in all media being however stimulated by yeast extract (fig. 52) in the presence of which, pigment formation is also more abundant and homogenous.

BURKHOLDER & MOYER (1943) studying a strain of *Trichophyton acuminatum* (*T. Sabouraudi*) showed it to be partially deficient for thiamin and that it was stimulated by liver or peptone. Similar results were obtained by the same authors with *Trichophyton sulfureum*. Our strain of *T. Sabouraudi* did not require any of the vitamins tested for growth, but it was stimulated by yeast extract (fig. 53), differing thus from that of the above mentioned authors. As to *T. sulfureum*, our strain showed entirely different behaviour to that observed by the same authors. It showed practically no growth in any of the media, except on the one containing yeast extract (fig. 54). Even though it was not shown to require any of the vitamins tested, yeast extract exerted a marked growth-promoting action.

OYAMA (1937) obtained with *Trichophyton crateriforme* (*T. tonsurans*) slow growth on a mineral medium, not stimulated by thiamin. However a concentrate of rice polishings showed a growth stimulating effect. Our results agree with the ones of this author, our strain not requiring any of the vitamins tested. Yeast extract had a marked growth stimulating action (fig. 55).

BURKHOLDER & MOYER (1943) showed that *Trichophyton violaceum* did not require thiamin for growth, which was markedly stimulated by peptone or liver concentrate. The growth of our strain was not affected by any of the vitamins tested, being nil, except on the medium containing yeast extract which had a marked growth-stimulating action (fig. 56).

Trichosporon Beigelii was shown by OYAMA (1937) to grow poorly in a mineral medium. Growth was not affected by the addition of thiamin, but rice polishings concentrate had a definite stimulating action. Our strain required thiamin and biotin, showing no growth on any of the media where these vitamins were not present. (fig. 57).

ARÊA LEÃO (1940) describing *Trichosporon minor* as a new species remarked its poor and slow growth in Czapek-Dox medium. Our strain is the original one described by that author. It did not grow on the basal medium nor on the one without thiamin (fig. 58), which confirms that it could not grow in Czapek-Dox medium. It showed good growth on the remaining media and it was not stimulated by yeast extract.

In our opinion the study of the nutrition and metabolism of pathogenic fungi is of great importance and interest, not only to bring a better knowledge to the biology of these microorganisms but also because it may be useful in establishing the use of culture media of definite chemical composition in which fungi of the most varied nutritional types may be grown. Once these media are obtained, which we believe may happen in the near future, the morphological study of these microorganisms would become more uniform, permiting the use, by various authors, of media with reproducible composition. Furthermore, the better knowledge of the nutrition of these microorganisms may provide useful criteria to be employed in their still uncertain classification. Several papers have already been published on this subject, among which those of WICKERHAM (1946) and WICKERHAM & BURTON (1948) on the assimilation of carbon and nitrogen compounds by yeasts, that of SCHULTZ, ATKIN & FREY (1940), with a tentative biochemical classification of a certain group of yeasts, and that of SCHOPFER (1944) on the genus *Candida*.

However, as emphasized by SCHOPFER & GUILLOUD (1945), caution is needed in the interpretation and application of the results, which may led to avoidable errors or exaggeration. In this connection, the work of MACKINNON & A.-ALLENDE (1948) is very suggestive; these authors studying five strains of *Trichophyton discoides* and two strains of *Trichophyton ochraceum* found three different types of vitamin deficiencies in the first and two in the second. Considering these two species as synonyms (CONANT *et al.* 1944; MACKINNON & A. ALLENDE, 1948), it is concluded that among seven strains studied, there are 4 different types of vitamin requirements. Obviously it would be absurd to admit on this basis the existence of four different species.

Our results are only considered valid for the strains used and under our experimental conditons; it is not possible to apply them to the various species, which show great morphological and physiological variation.

Our future research is planned towards the detailed study of the nutrition and vitamin requirements of each group, genus, species or strain. Better knowledge in this field is required for the various groups of pathogenic fungi. The data so obtained may bring light to many so far obscure problems related to the mode of infection, pathogenicity and natural habitat of these microorganisms (NICKERSON, 1948).

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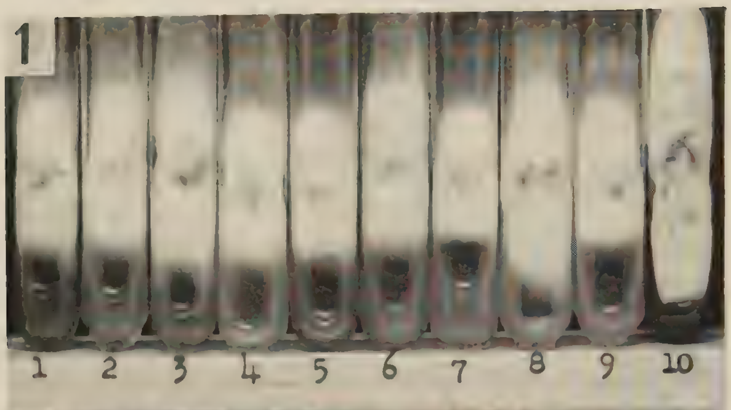
Chave para a identificação dos meios

Tubo 1 — Meio basico sem vitaminas.
Tubo 2 — Meio basico + todas as vitaminas, exceto tiamina.
Tubo 3 — Meio basico + todas as vitaminas, exceto riboflavina.
Tubo 4 — Meio basico + todas as vitaminas, exceto piridoxina.
Tubo 5 — Meio basico + todas as vitaminas, exceto acido nicotinico.
Tubo 6 — Meio basico + todas as vitaminas, exceto pantotenato de calcio
Tubo 7 — Meio basico + todas as vitaminas, exceto inositol.
Tubo 8 — Meio basico + todas as vitaminas, exceto biotina.
Tubo 9 — Meio basico + todas as vitaminas.
Tubo 10 — Meio basico + todas as vitaminas + extrato de levedura.

Key to the identification of the media

Tube 1 — Basal medium without vitamins.
Tube 2 — Basal medium + all the vitamins, except thiamin.
Tube 3 — Basal medium + all the vitamins, except riboflavin.
Tube 4 — Basal medium + all the vitamins, except pyridoxin.
Tube 5 — Basal medium + all the vitamins, except nicotine acid.
Tube 6 — Basal medium + all the vitamins, except calcium pantothenate
Tube 7 — Basal medium + all the vitamins, except inositol.
Tube 8 — Basal medium + all the vitamins, except biotin.
Tube 9 — Basal medium + all the vitamins.
Tube 10 — Basal medium + all the vitamins + yeast extract.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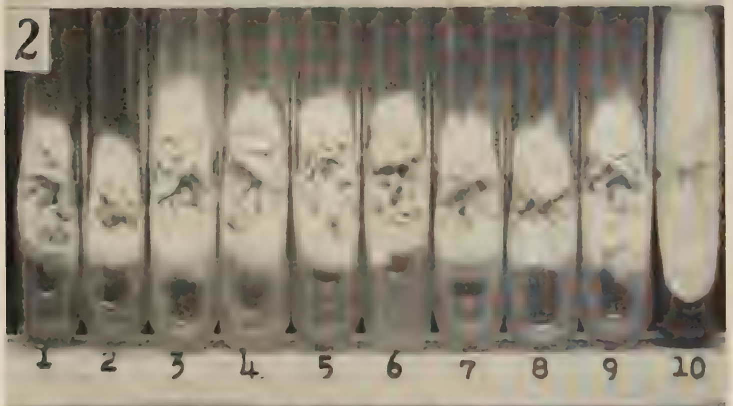
Achorion gallinae
1456 (50 days).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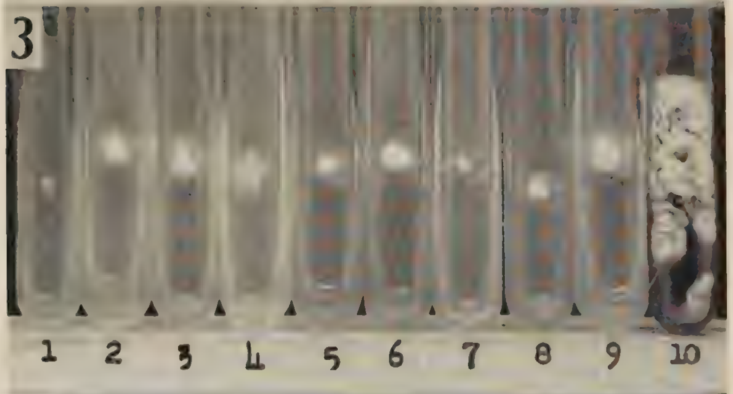
Achorion gypseum
1439 (50 days).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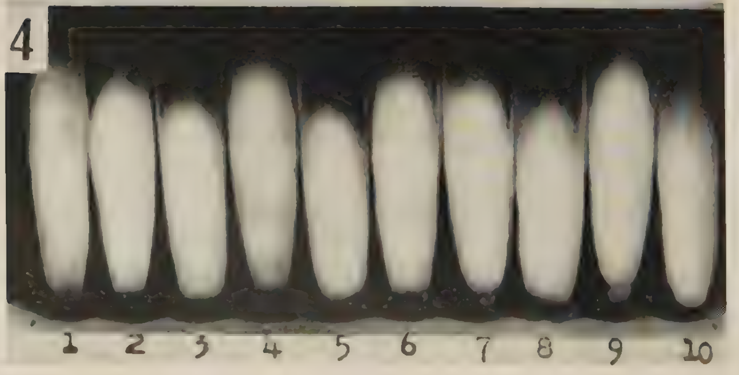
Achorion Schoenleinii
1425 (50 days)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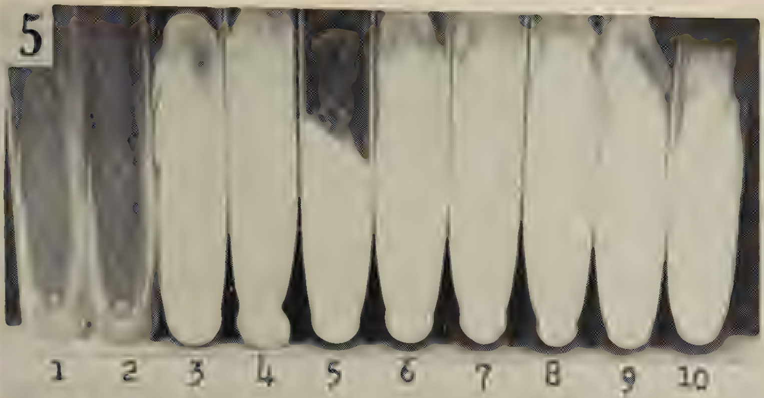
Acremoniella Lutzi
866 (60 days).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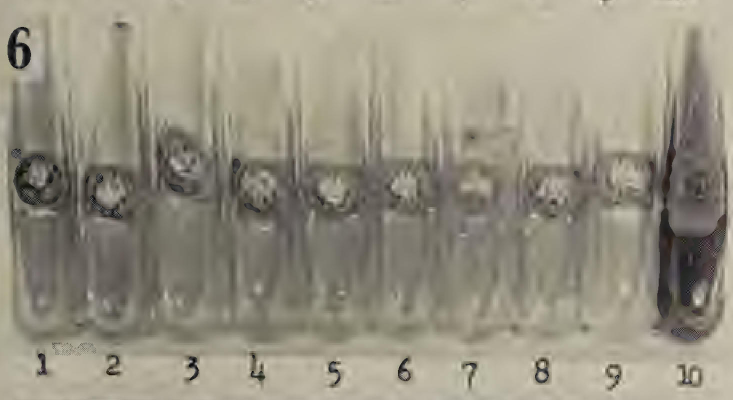
Acremonium Potronii
1610 (60 days).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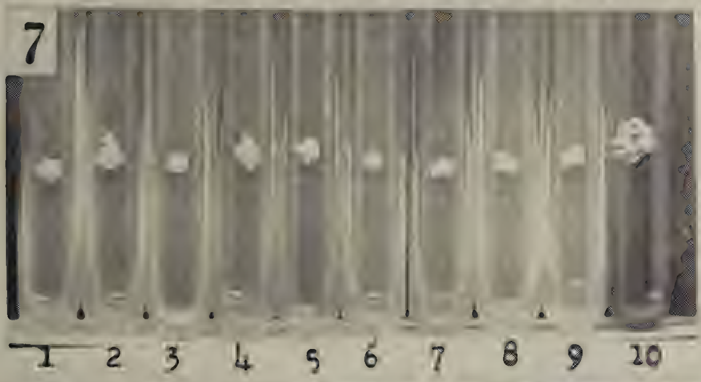
Actinomyces brasiliensis
2590 (60 days).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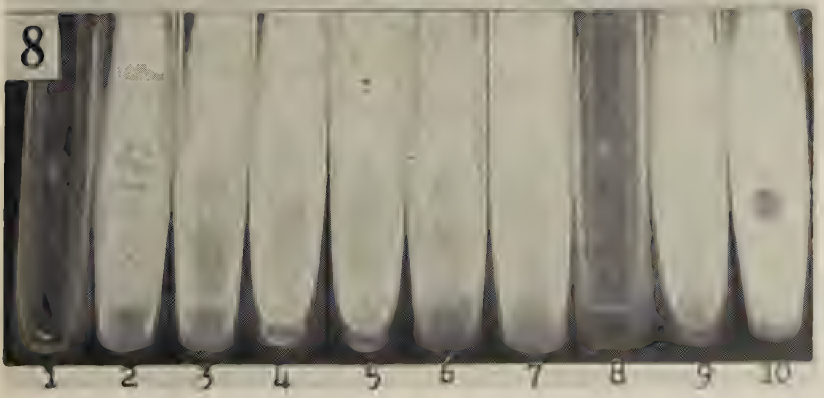
Actinomyces madurae
2593 (50 days).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Allescheria Boydii
386 (21 days).

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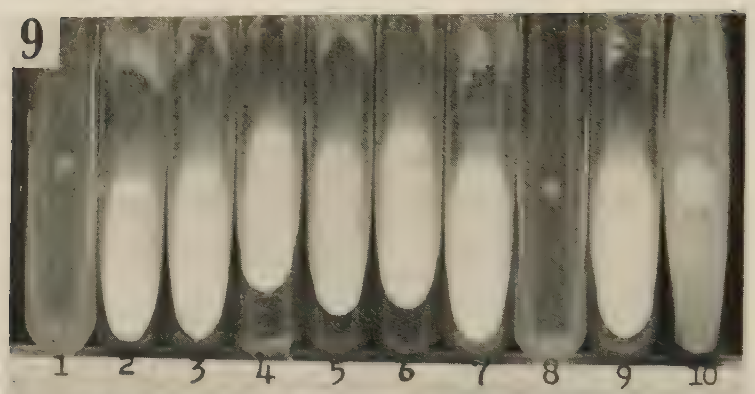
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Allescheria Boydii
1699 (50 days).

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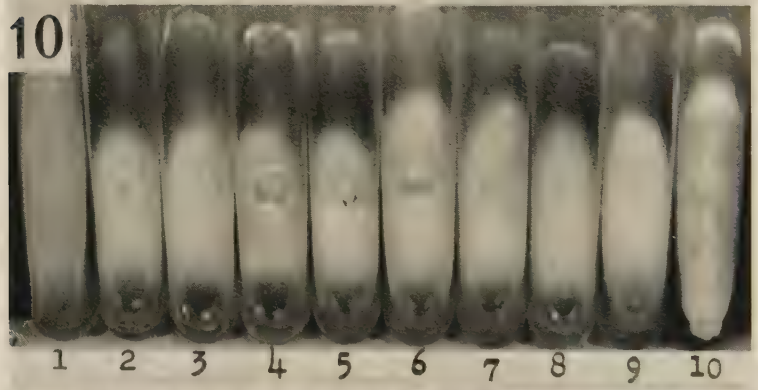
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Blastomyces dermatitidis
1901 (60 days).

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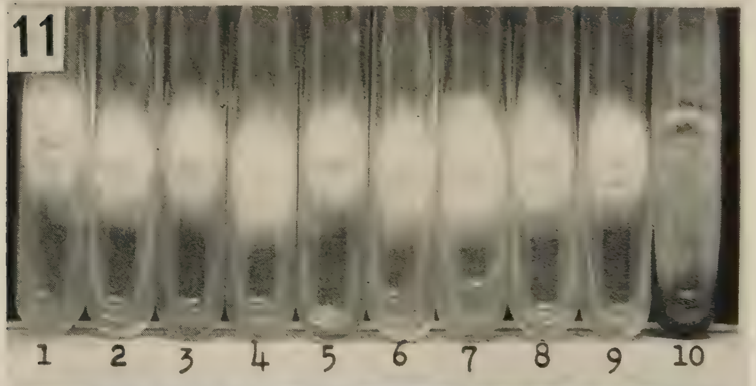
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Blastomyces dermatitidis
1902 (50 days).

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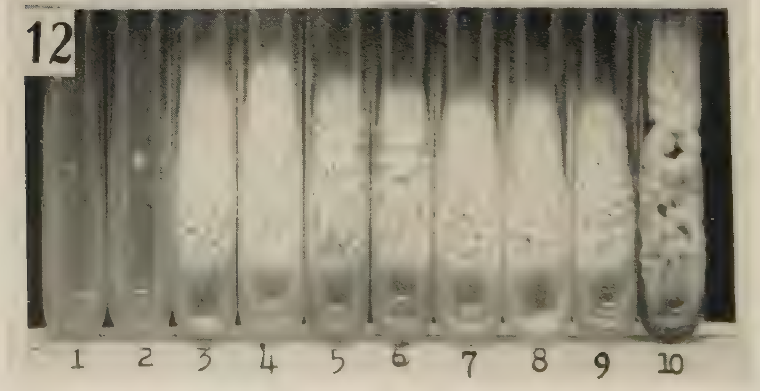
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Candida suaveolens
2032 (50 days).

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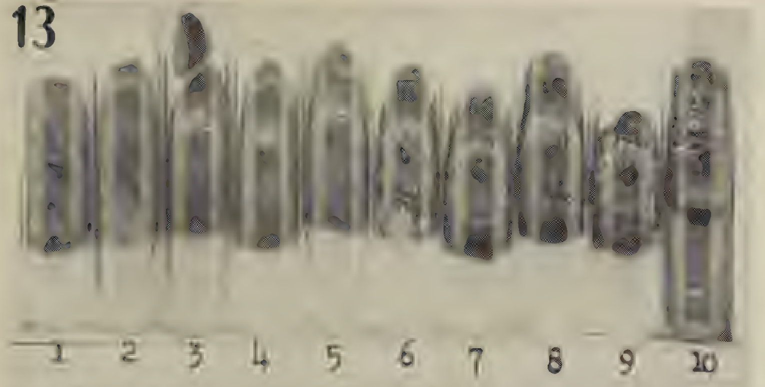
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Cladosporium Wernecki
1886 C (50 days).

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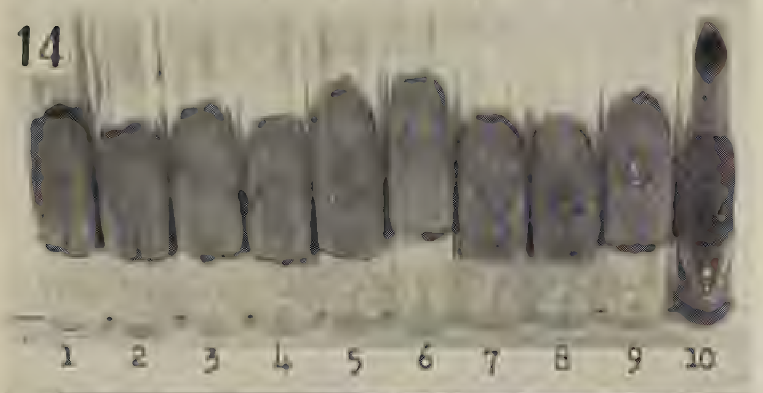
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Cladosporium Wernecki
1886 M (60 days).

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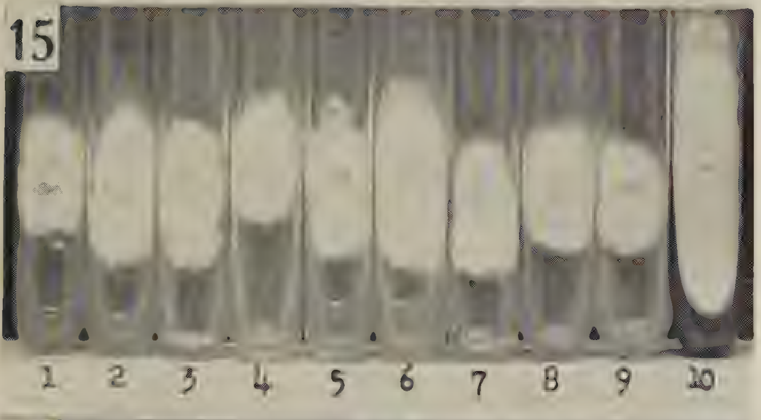
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Coccidioides immitis
1893 (50 days).

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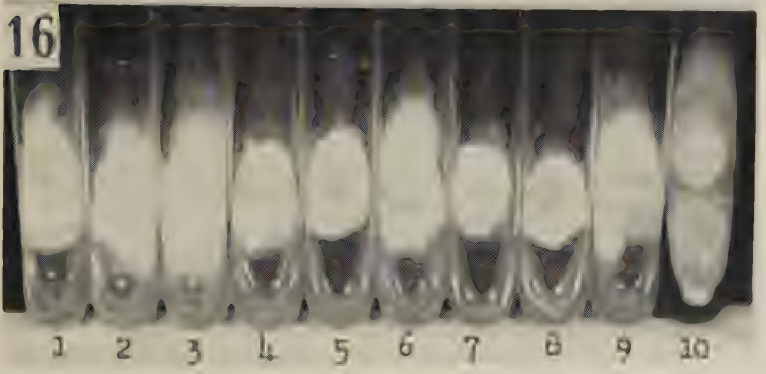
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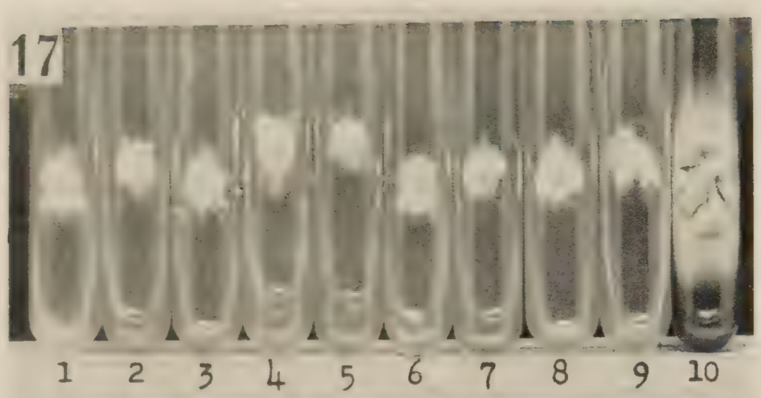
Coccidioides immitis
1894 (60 days).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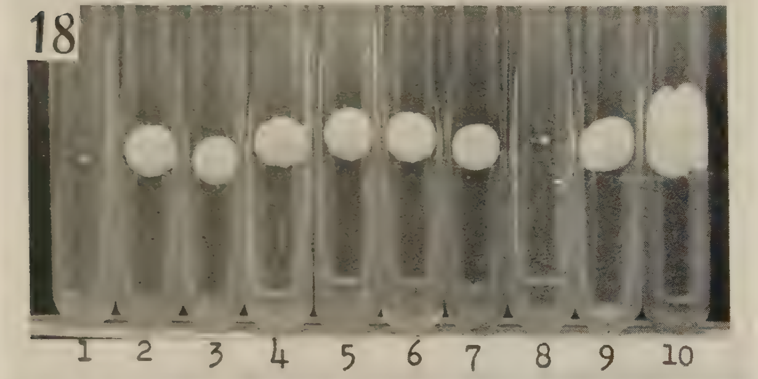
Coccidioides immitis
1897 (50 days).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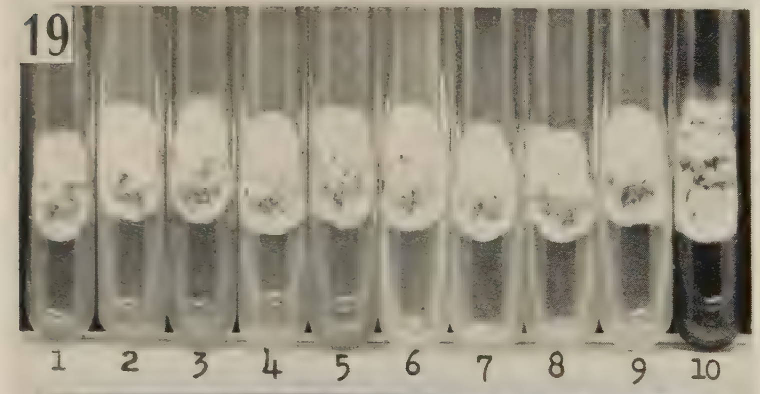
Debaryomyces Hudeloi
1940 (21 days).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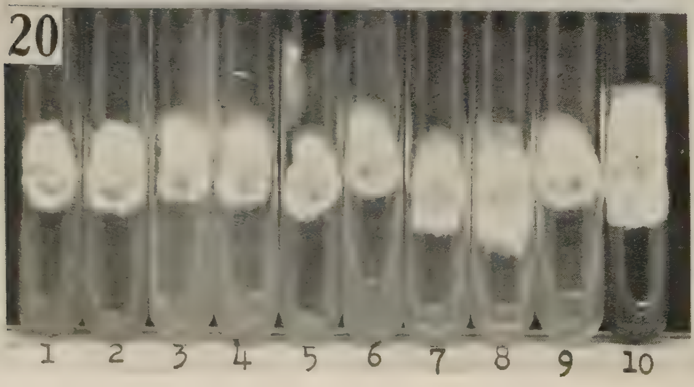
Endodermophyton
concentricum
1176 (50 days).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Endodermophyton Roquettei
1061 (50 days).

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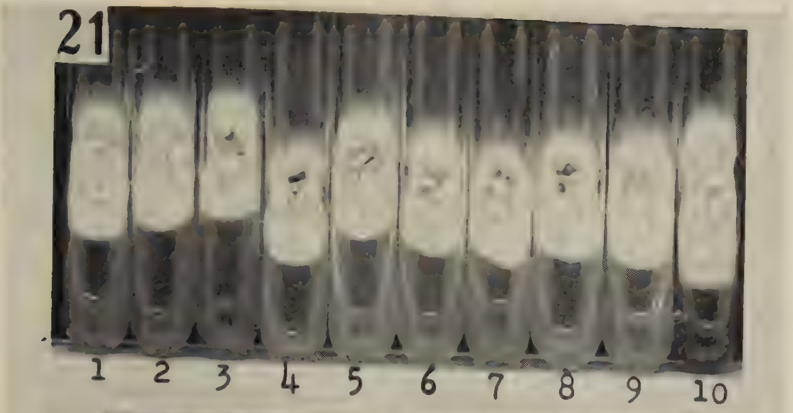
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Endodermophyton tropicale
1718 (50 days).

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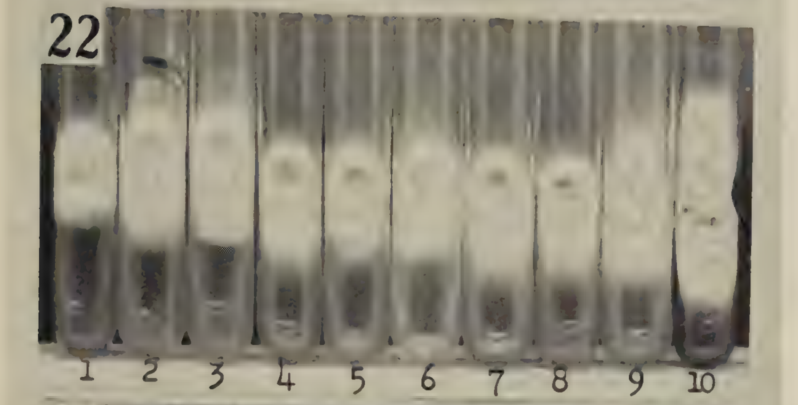
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Epidermophyton floccosum
1923 (50 days).

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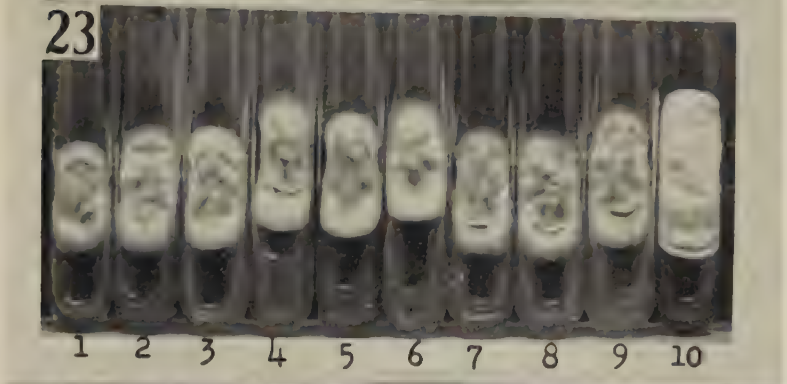
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Glenosporella Loboi
1488 (50 days).

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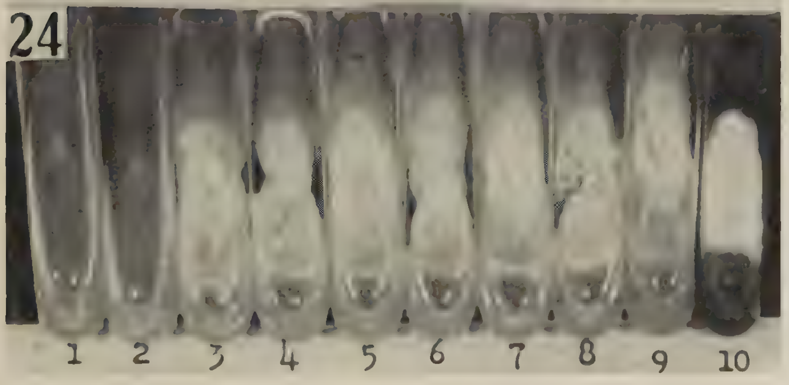
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Glenosporopsis amazonica
1848 (60 days).

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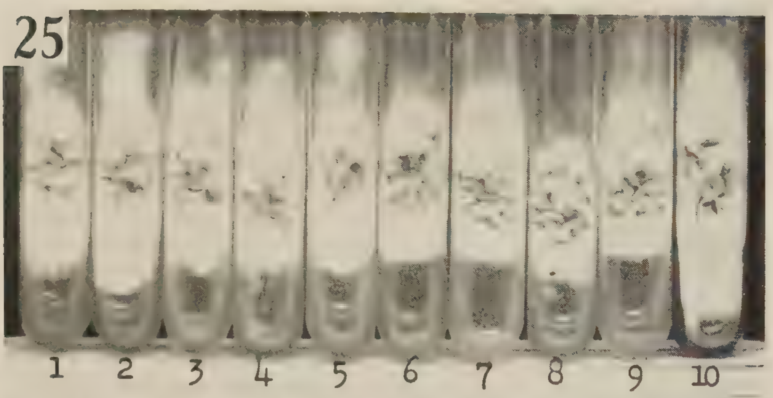
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Haplosporangium parvum 2606 (50 days).

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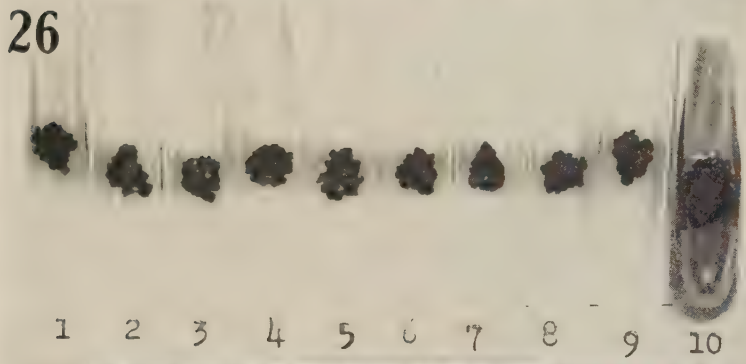
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Hemispora stellata 1125 (60 days).

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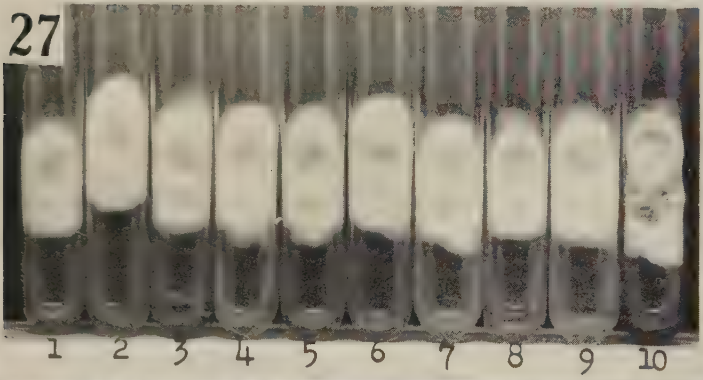
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Histoplasma capsulatum 954 (50 days).

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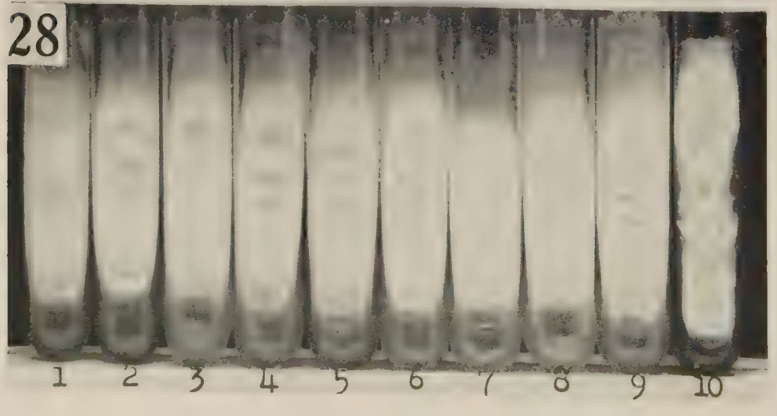
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Histoplasma capsulatum 1860 (50 days).

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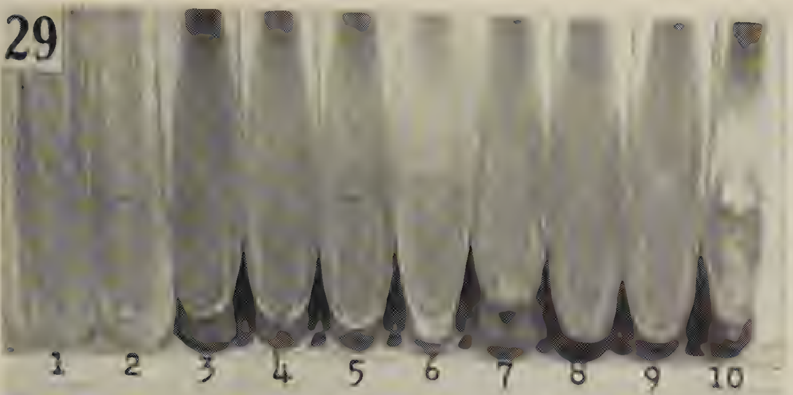
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Histoplasma capsulatum
2357 (60 days).

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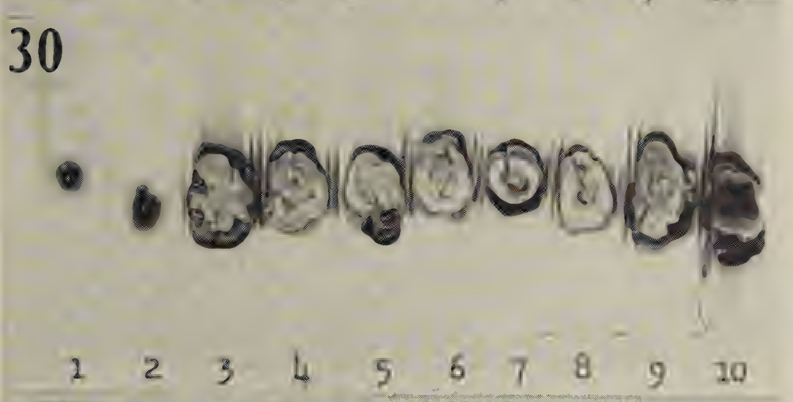
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Hormiscium dermatitidis
1800 (60 days).

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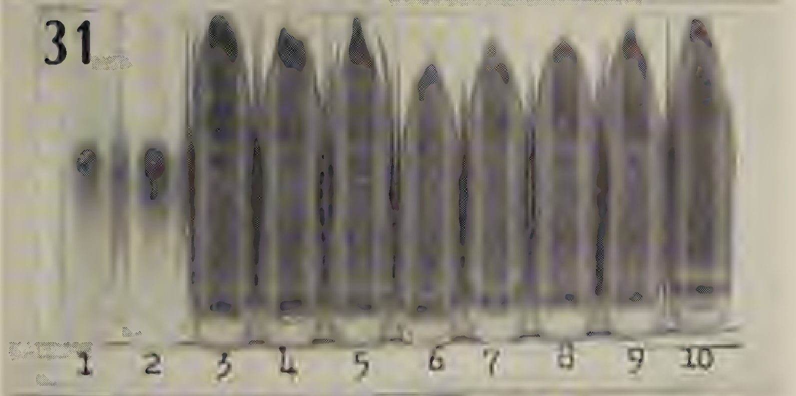
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Hormodendrum algeriensis
2221 (60 days).

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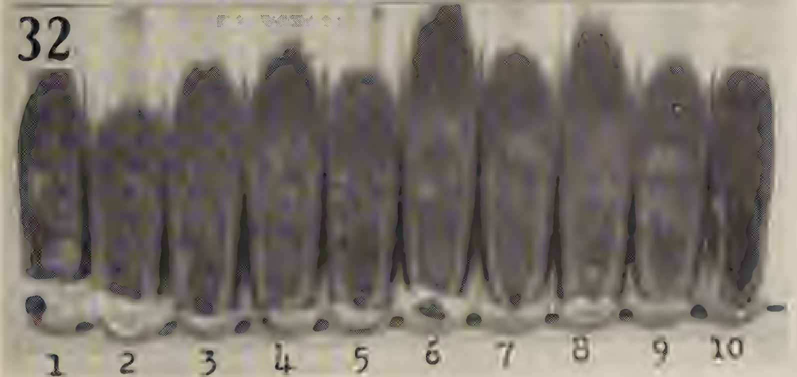
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Hormodendrum Langeroni
990 (60 days).

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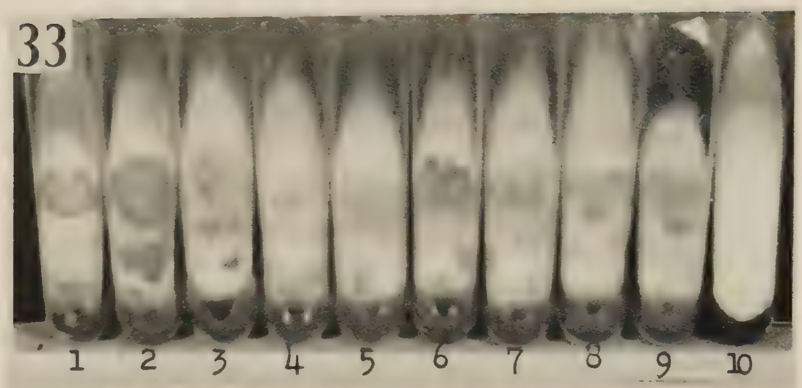
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Madurella americana
1182 (60 days).

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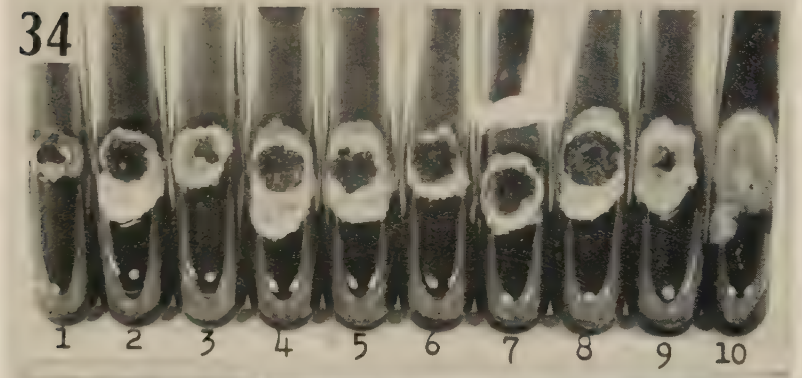
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Madurella Ikedai
2583 (60 days).

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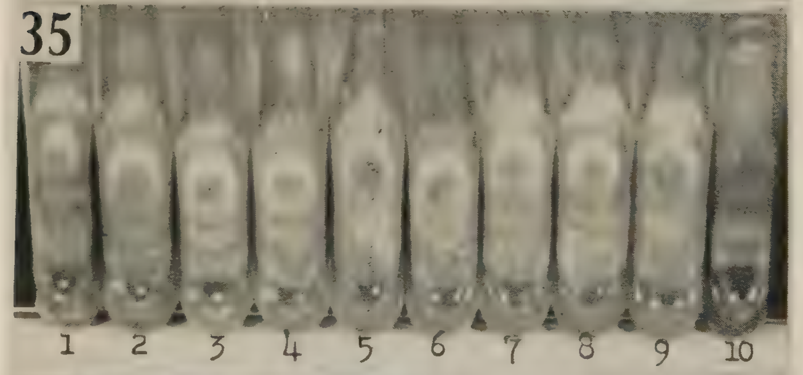
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Madurella mycetomi
2372 (60 days).

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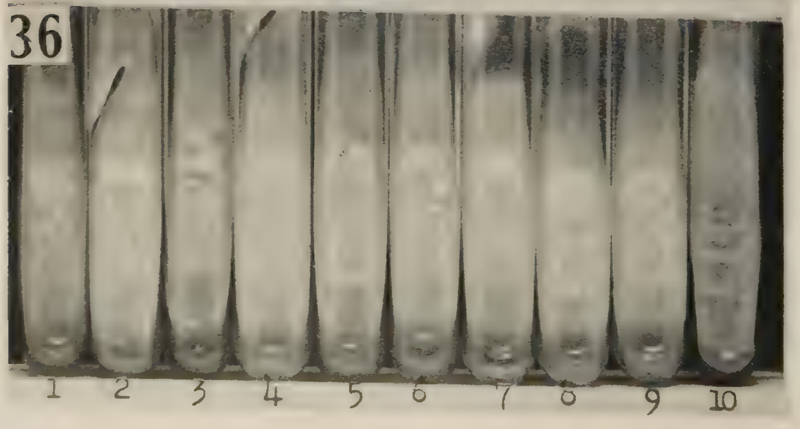
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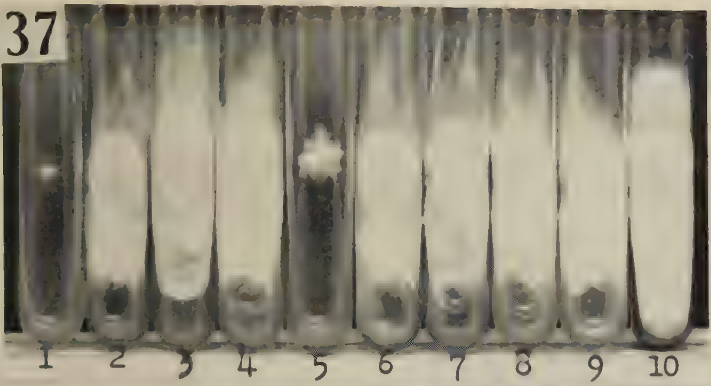
Malassezia tropica
2430 (50 days).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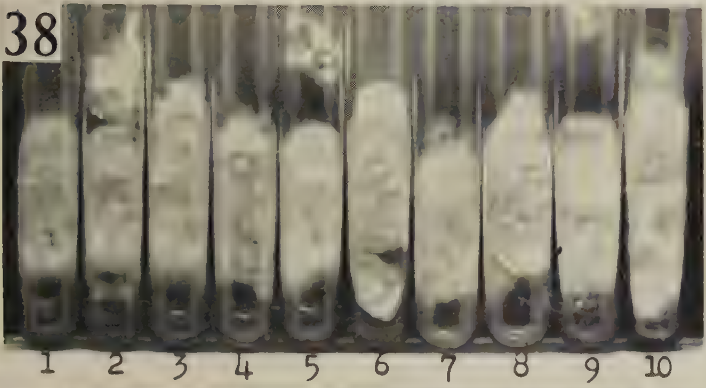
Microsporum Audouini
1447 (50 days).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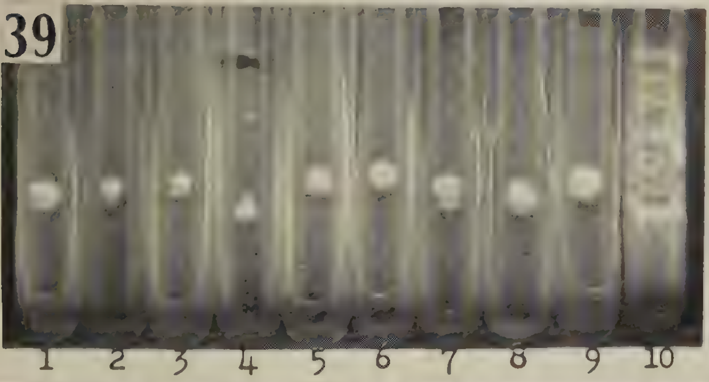
Microsporum canis
2555 (50 days).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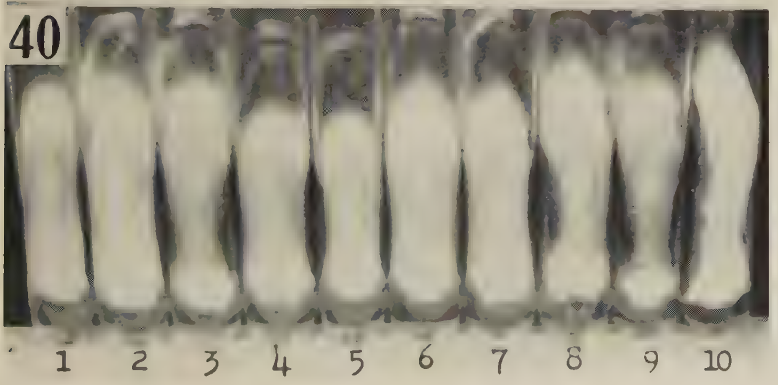
Microsporum ferrugineum
569 (50 days).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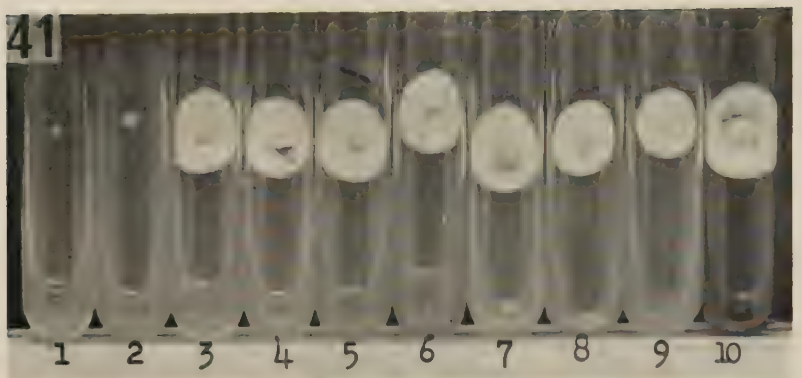
Monosporium apiosperum
1603 (60 days).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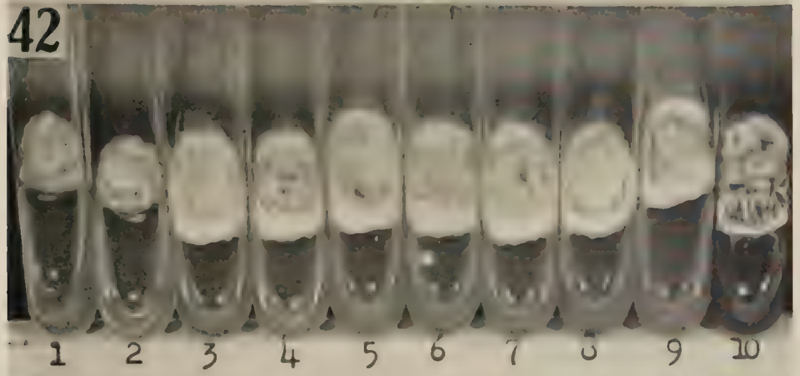
Paracoccidioides brasiliensis 2596 (50 days).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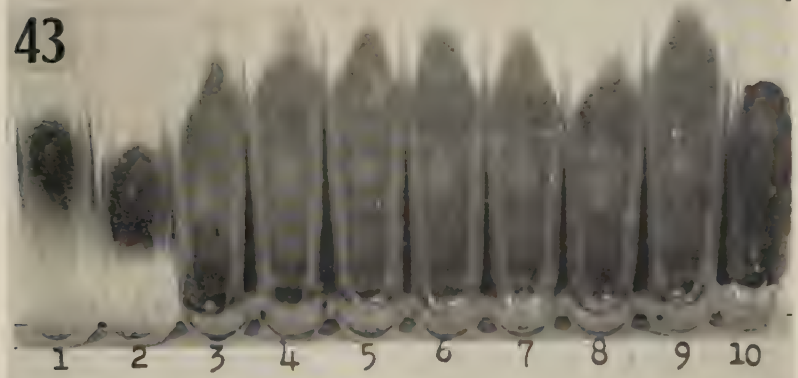
Paracoccidioides brasiliensis 2597 (60 days).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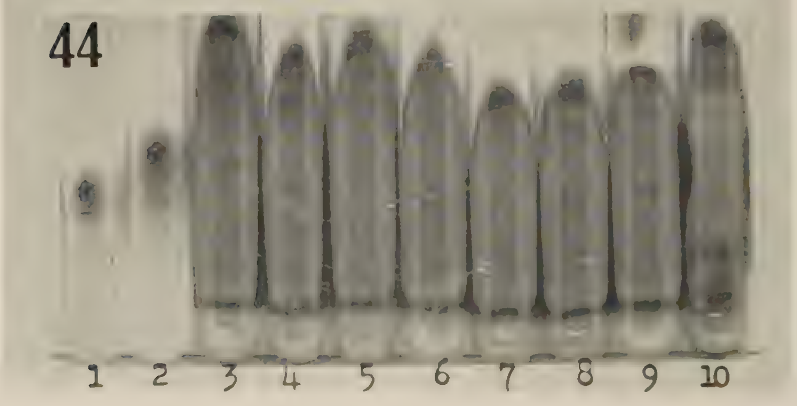
Phialophora compactum 1991 (60 days).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Phialophora Jeanselmei 2000 (50 days).

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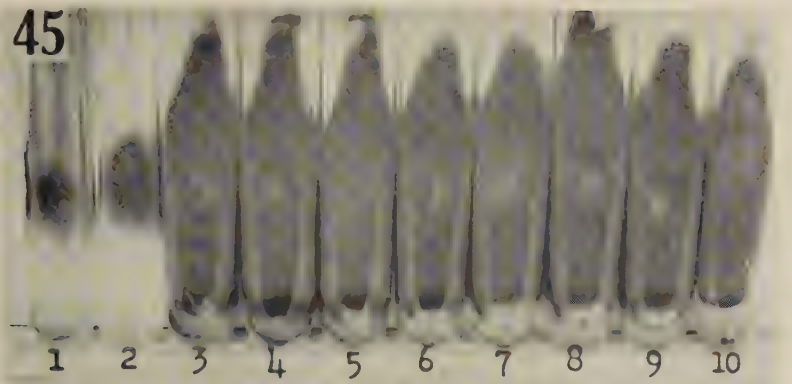
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Phialophora Pedrosoi
1885 (60 days.)

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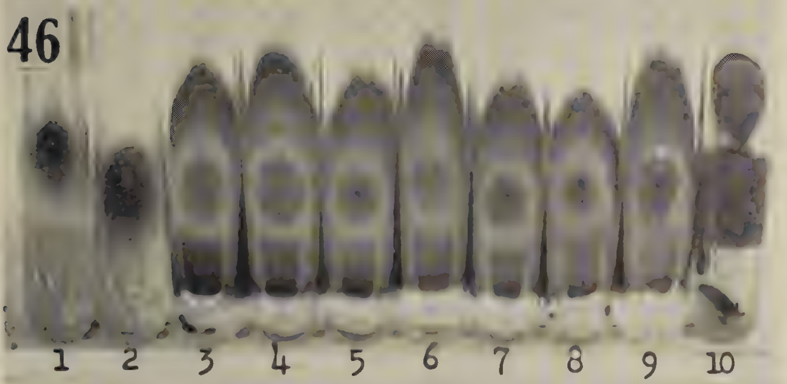
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Phialophora verrucosa
1995 (60 days).

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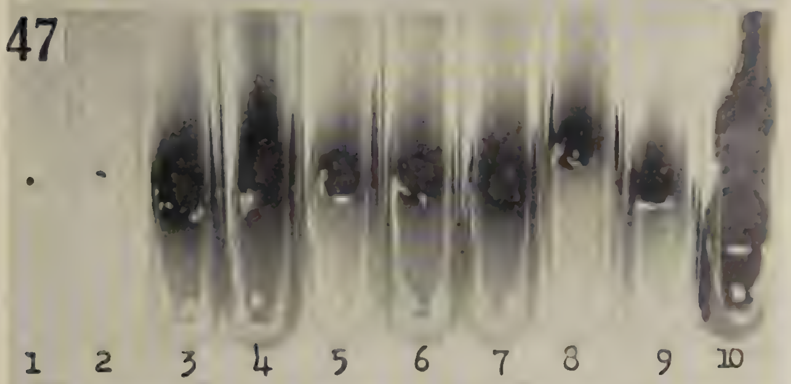
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Piedraia Hortai
1538 (60 days).

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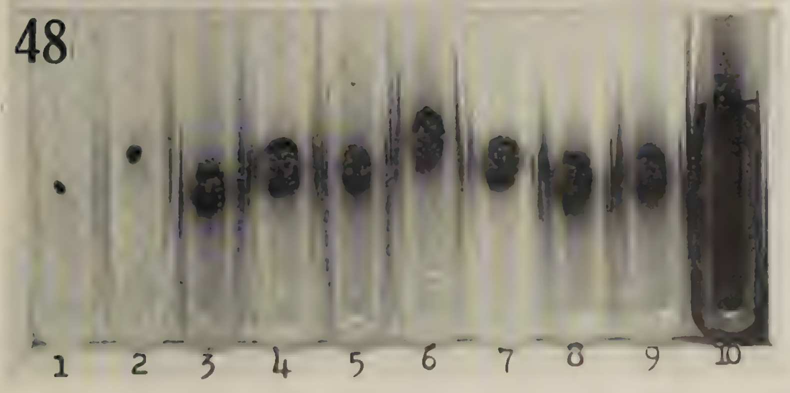
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Piedraia Hortai
2458 (50 days

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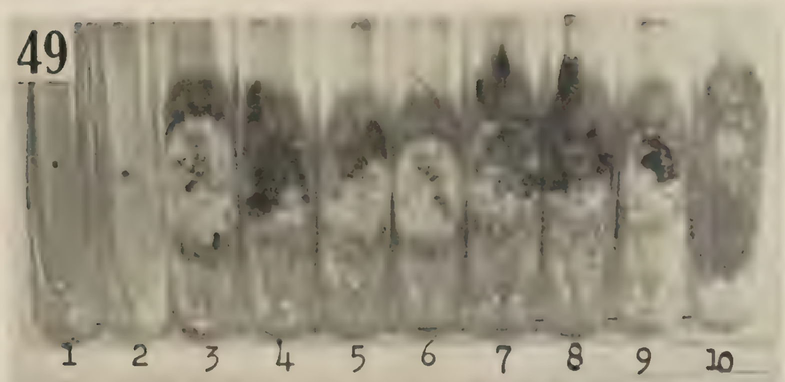
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Sporotrichum Schenckii 2562 (60 days).

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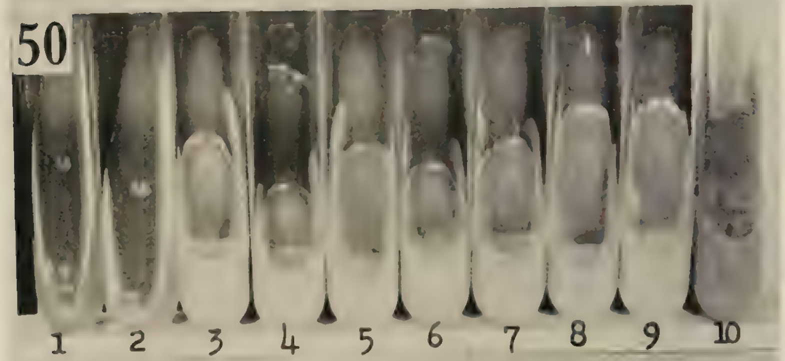
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Torulopsis neoformans 2561 (60 days).

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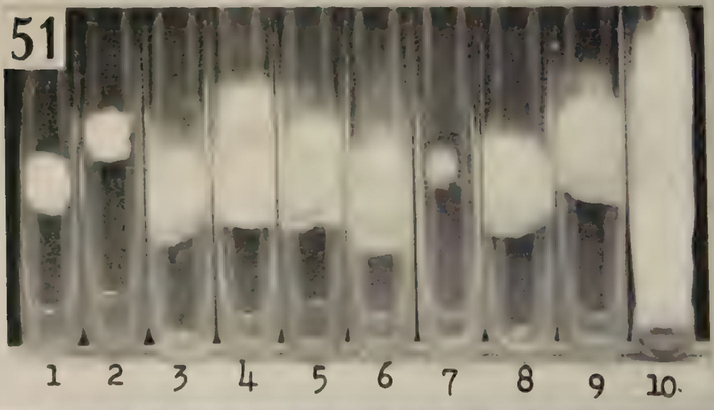
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Trichophyton mentagrophytes 2543 (50 days).

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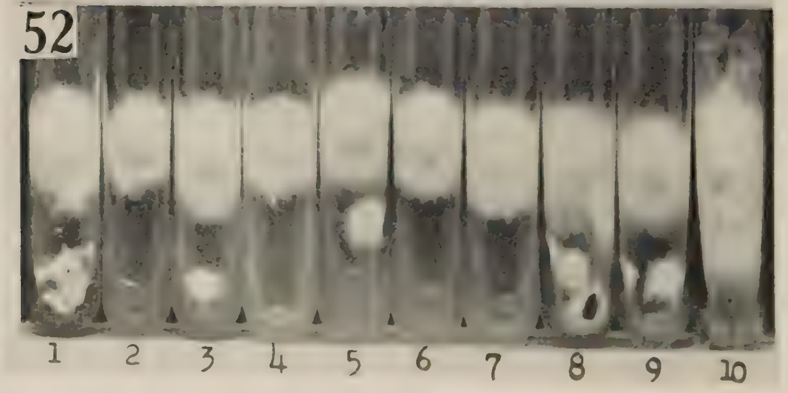
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Trichophyton rubrum 2553 (50 days).

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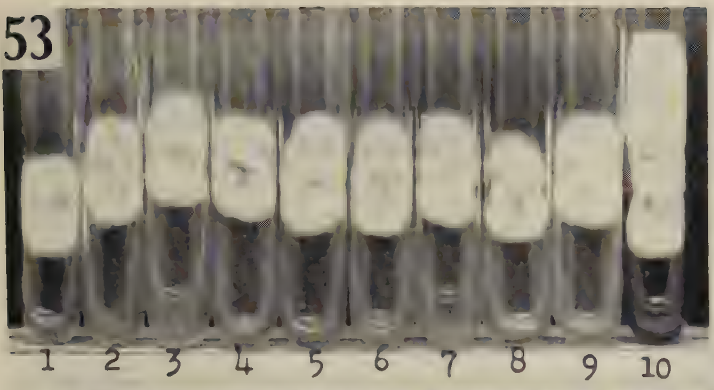
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Trichophyton Sabouraudi
512 (50 days).

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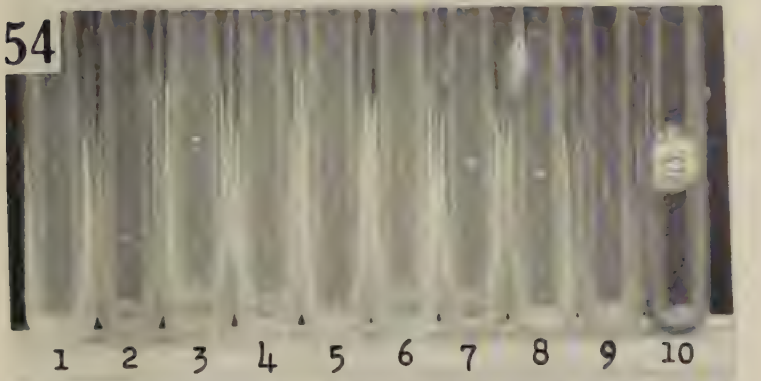
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Trichophyton sulfureum
1421 (50 days).

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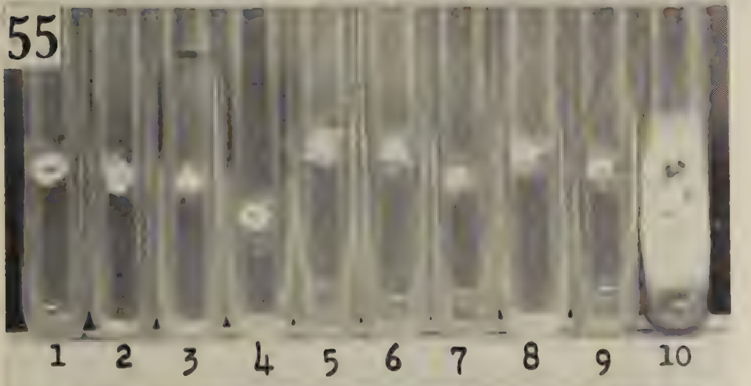
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Trichophyton tonsurans
612 (50 days).

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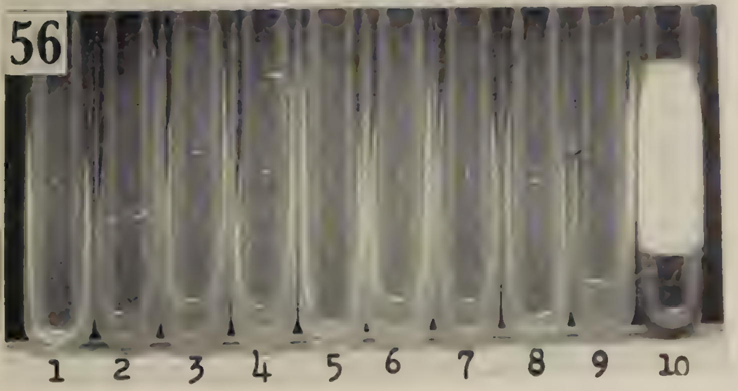
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Trichophyton violaceum
1254 (50 days).

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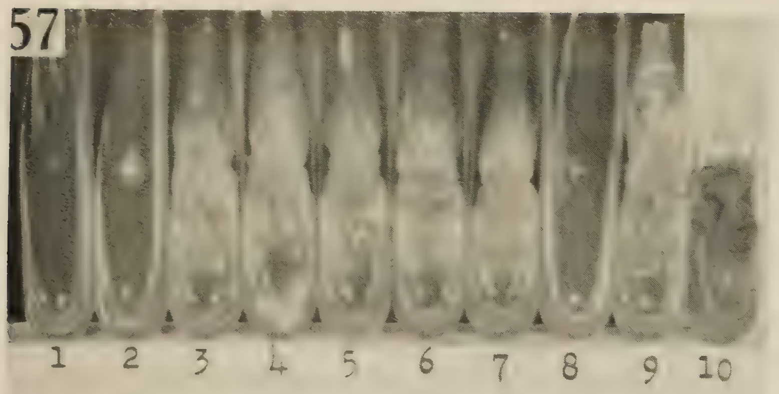
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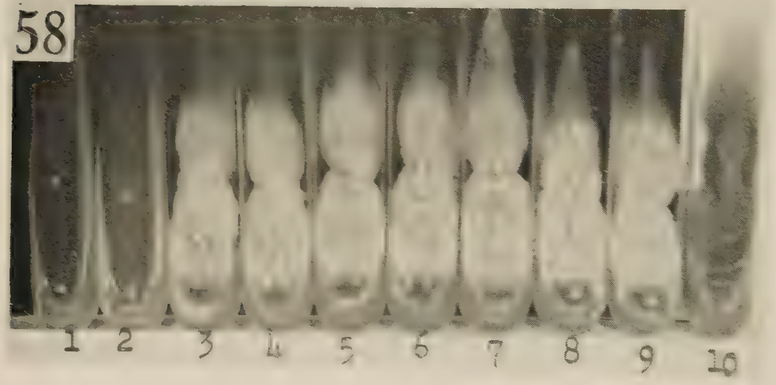
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Ten years of investigation about mycopathology in Portugal (1938–1948)

A. LIMA CARNEIRO

(20. 5. 1949.)

The researches about mycopathology began in Portugal in 1862 with Prof. MAY FIGUEIRA (a).

Then, other authors studied the medical mycology (b): EDUARDO PIMENTA, LUÍS DE FREITAS VIEGAS, ANTÓNIO RAMALHO, PACHECO DE MIRANDA, CHARLES LEPIERRE, FROILANO DE MELO, ANTÓNIO PAES, LORETO DE SOUSA, ARMANDO FERREIRA ÁLVARES, LUIS GONZAGA FERNANDES, J. F. ANTÓNIO FERNANDES, J. A. DE CARMO VAZ, LIMA CARNEIRO, NOGUEIRA MARTINS, VILAS-BOAS NETO, REBELO ROCHA, ÁLVARO LAPA, SANT'ANA PAIS, LEITE NORONHA, CÉSAR MARTINS, ARNALDO RODRIGUES, NEVES SAMPAIO, LOBATO DE FARIA, JORGE MASCARENHAS e MANUEL CARRASCO.

Among these investigators, Prof. FROILANO DE MELO deserves a special place, as in 1917 he gave particular increase to the studies of mycosis, and in 1921, in the Portuguese Continent, he got to assemble a good number of investigators who published several studies about mycology.

Later on, other investigators (c) were devoted to the same matter.

Now, I will speak but about the Portuguese publications brought out between 1938 and 1948 (d).

In 1938 — LIMA CARNEIRO (1) (*), through a public lecture, discoursed on the scall-head's problem.

After speaking of the illness and its agents, he made a few considerations about the scall-head both in literature and ethnography, described the old modern treatment, and proposed several means for struggling with those dermatomycosis.

J. M. PALMEIRO (2), after referring to scall-head and the practise of the cultures' essays identified some trichophytosis he found in the horse, ox, dog, cat, rabbit and mouse.

J. A. PIRES DE LIMA (3) treated of the scall-head too, its contagion and treatment.

MORAIS CARDOSO (4), after several considerations about the maduromycosis of black grain, described the special case of a boy whom he studied both clinically and under the anatomical and pathologycal point of view.

LIMA CARNEIRO (5) studied the scall-head in the scholar age, showed the necessity of struggling with it and suggested the convenient treatment.

The same author (6) pointed the first works printed in Portugal about

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(*a*) MAY FIGUEIRA — *Planta criptogâmica da ordem dos cogumelos do género Arpergillus, espécie glaucus (Fries), achada no pulmão humano*, in ,,Jornal da Sociedade das Ciências Médicas'', Lisbon, 1862.

(*b*) Vid. A. LIMA CARNEIRO — *As micoses em Portugal* (Revista crítica) — Oporto 1948.

(*c*) Vid. A. LIMA CARNEIRO — *Op. cit.*

(*d*) TILL 1938, 71 works about mycopathology were published.

*) The numbers mentioned in the text refer to the Bibliography, at the end of this work.

mycology, the first investigations about dermatomycosis and parasites, and the localities where such diseases were first found out in Portugal.

He presented a map, with the geographical distribution of dermatophytes and a table with their distribution according to the parts of the body attacked.

This author published a long medical mycological Portuguese Bibliography.

VICENTE XAVIER LOBO (7) described the species *Scopulariopsis Sehnsuchta* Melo; he made reference to the mycological elements and the characters of the cultures in various means.

EUGENIO TROPA and SOUSA DIAS (8), in *Notas Parasitológicas* dealt in some Fungus-parasites of domestic animals, found at Oporto and Aveiro.

NEVES SAMPAIO (9), after noting the geographical distribution of dermatophites, described a case of twined herpes due to the *Microsporum felineum* and four other cases of tonsure scall-head caused by *Tr. violaceum*.

SÁ PENELA and JUVENAL ESTEVES (10) made several considerations about the geographical distribution of dermatophytes.

WOHLWILL and MORAIS DAVID (11) describe a case of Darling histoplasmosis which attained a woman 59 years old. The etiology of the disease was revealed by the histological essay. The authors call the attention on this case, the first described in Portugal, where certainly others will be found.

LIMA CARNEIRO (12) presented a work about the scall-head in the popular tradition and Portuguese literature. Other works, already published about the matter, are all therein noted together with other elements further gathered.

JOAQUIM SALVADOR VALENTE (13) made a study about the botriomycosis.

LIMA CARNEIRO (14) studied the *Trichophyton violaceum* and its frequent existence in Portugal, and concluded that 42,8 % of the dermatomycosis already studied in Portugal are provoked by this fungus.

SALAZAR LEITE and JORGE HORTA (15) published a study on the inoculations of levuriform fungus in animals.

PINTO NOGUEIRA (16) made a brief revision of some of the studies already published about the scall-head; he treated the followed technic and the materials assembled, reported the frequency of the *Trichophyton violaceum* and low percentage of the *Tr. acuminatum* in Lisbon, and pointed the towns where a greater number of cases of that dermatomycosis can be found.

SÁ PENELA and JOSÉ RODA (17) occupied their activity with 494 cases of scall-head, observed at the „Hospital do Desterro" where the predominance of the *Trichophyton violaceum* was also noticed.

They mention the good results of X-rays for this illness-treatment.

LIMA CARNEIRO (18) described a case of sycosis produced by two fungi: *Trichophyton violaceum* Bod. and *Trichophyton glabrum* Sab.

MANUEL FERREIRA and LIMA CARNEIRO (19) presented the result of a series of observations on a few phenomena of antibiosis in fungi. The authors verified that the doughs *Rhodotorula glutinis* var. *lusitanica*, *Saccharomyces cerevisiae* Han. and another not identified inhibited, in solid means, the development of the *Trichophyton crateriforme* Sab.

ARNALDO TANISSA (20), after a great deal of considerations regarding the hipovitaminose A in the patients suffering from dermatomycosis shows the results obtained by the employment of a solution of vitamin A into cod-fish liver oil and only with cod-fish liver oil in the dermatomycosis and concluded that such favourable results are due to the fat acids of the cod-fish liver oil. From 22 cases, he was well succeeded in 17 cures, 3 reliefs and 2 unsuccessful cases. He also dealt in the topic therapeutic used by the Americans.

MANUEL TENDER (21) described a case of glossitis probably of actinomycosic origin.

Maria dos Santos Pinto (22) dealt in a case of mycosis localized at the vulva and a finger. She made the microscopical examination of the lesion scales but did not identify the parasite. She reported the followed treatment and made her indeference.

Arnaldo Tanissa made a few experiments at the laboratory as to the fungistatic action of the cod-fish liver oil, by employing several dermatophytes.

The concentration 10 % proved quite fungistatic for every fungus employed in the referred experiments. The concentration 1 % only paralised the development of a few.

Besides, he determined the duration of necessary exposing in order to kill the cultures of the same fungi. This author (24) reports 35 works he took advice of, published in 1945, on the local treatment of the scall-head and the results obtained.

Lima Carneiro (25) made a detailed reference to the studies published in Portugal about mycosis, since 1862, and gave us a long myco-pathological bibliography.

Portuguese medical mycological bibliography

1) 1938, A. Lima Carneiro — *Uma Miséria Social— As Tinhas* — Public Lecture at ,,Liga Portuguesa de Profilaxia Social'' on the 22nd.-IV-1938, in ,,Conferências da Liga Portuguesa de Profilaxia Social'' — 6th series — Oporto.

2) 1938, J. M. Palmeiro — *Contribuição ao estudo cultural e experimental das tinhas e identificação de algumas das existentes em Portugal,* in ,,Repositório de Trabalhos do Lab. Central de Patologia Veterinária'', vol. IV, Lisbon.

3) 1938, J. A. Pires de Lima — *As Tinhas,* in ,,A Voz de Fátima'', 13th. June, and ,,Palavras de um Médico'', vol. I.

4) 1938, Morais Cardoso — *Maduromicose de grãos pretos* (primeiro caso português autóctono) in ,,Lisboa Médica'', vol. XVI.

5) 1940, A. Lima Carneiro — *A Tinha — Meios de combater* — in ,,A Saúde Escolar'', n° 36, VIth year.

6) 1942, A. Lima Carneiro — *Dermatófitos Portugueses* — Copy from n° 301 of ,,Revista de Medicina Veterinária'' and copy of the 2nd vol. of ,,Actas do Congresso Nacional de Ciências Naturais'', Lisbon.

7) 1942, Vicente Xavier Lobo — *Scopulariopsis Sehnsuchta de Melo 1932, n'est qu'une espèce saprophyte,* in ,,Arquiv. da Escola Médico-cirúrgica de Nova-Goa'', series A, Fasc. 16.

8) 1943, Eugénio Tropa e Sousa Dias — *Notas parasitológicas* — Subsídios para a Corografia parasitológica do Norte, in ,,Rev. de Med. Veterinária'', n° 306.

9) 1943, Neves Sampaio — *Cinco casos de Dermatomicoses,* in ,,Amatus Lusitanus'', vol. II, n° 8.

10) 1943, Sá Penela e Juvenal Esteves — *Tratamento das Tinhas* (Service n° 3, ,,Hospital do Desterro''), in ,,Amatus Lusitanus''.

11) 1943, Wohlwill and Morais David — *Histoplasmosis de Darling* — in ,,Lisboa Médica''.

12) 1944, A. Lima Carneiro — *A Tinha na Tradição Popular e na Literatura Portuguesa,* in ,,Jornal do Médico''.

13) 1944, Joaquim Salvador Valente — *A Botriomicose,* in ,,Rev. de Medicina Veterinária'', n° 309.

14) 1944, A. Lima Carneiro — *O Trichophyton violaceum Bod. — A sua frequência em Portugal* — Copy from ,,Boletim da Soc. Broteriana'', vol. XIX, 2nd series.

15) 1945, A. Salazar Leite e Jorge Horta — *Resultados de inoculações de animais de Laboratório de estirpes de fungos levuriformes,* in ,,Anais do Instituto de Medicina Tropical'', vol. II.

16) 1945, J. P. Pinto Nogueira — *Tinhas de Lisboa,* in ,,Anais do Instituto Tropical'', vol. II.

17) 1946, Sá Penela e José Roda — *Estatística das Tinhas dos anos de* 1943 *e* 1944 — Communication to the ,,Sociedade Portuguesa de Dermatologia e Sifiligrafia''.

18) 1946, A. Lima Carneiro — *Um caso de Sicose produzida pelo Trichophyton violaceum Bod. e pelo Trichophyton glabrum Sab.* Copy from ,,Portugal Médico'', vol. XXX, n° 5.

19) 1946, Manuel Ferreira e A. Lima Carneiro — *Fenómenos de Antibiose entre Fungos* — Observações de culturas em meios sólidos. Copy from ,,Boletim da Sociedade Broteriana'', vol. XX — 2nd series.

20) 1946, Arnaldo Tanissa — *Vitamina A. Óleo de Fígado de Bacalhau e Dermatomicoses,* in ,,Amatus Lusitanus'', vol. V, n° 6—7.

21) 1947, Manuel Tender — *Um caso de glossite actinomicósica,* in ,,Jornal do Médico'', n° 230.

22) 1947, Maria dos Santos Pinto — *Um caso de micose vulvar,* in ,,Jornal do Médico'', 1st Nov.

23) 1947, Arnaldo Tanissa — *Estudo da actividade fungiestatica e fungicida in ,,vitro'' do óleo de fígado de bacalhau,* in ,,Amatus Lusitanus'' — 6-463.

24) 1948, Arnaldo Tanissa — *O Tratamento local das Tinhas tonsurantes,* in ,,Gazeta Médica Portuguesa'', vol. I.

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Pierre–Ernest Pinoy (1873–1948)

par

J. Magrou
de l'Institut Pasteur de Paris

(30. 5. 1949)

Le 31 octobre 1948 disparaissait un éminent mycologue français, le Professeur Pierre-Ernest Pinoy, qui s'illustra dans une voie où l'Ecole italienne devait réaliser d'importantes découvertes ,celle de la Mycologie médicale. Mais là ne se borna pas l'activité de Pinoy, et nous voudrions, dans cette brève notice, esquisser l'ensemble de son œuvre et en faire ressortir la portée générale.

Ernest Pinoy est né à Paris, le 3 février 1873. Après ses études classiques au Lycée Charlemagne, il devint, en 1893, licencié ès-sciences physiques et, l'année suivante, licencié ès sciences naturelles. De 1897 à 1900, il fut préparateur du professeur Cornil à la Faculté de Médecine et acquit dans son laboratoire une connaissance approfondie de l'anatomie pathologique. Docteur en médecine en 1899, avec une thèse sur ,,La glande sous-maxillaire et la tuberculose'', il entra en 1901 à l'Institut Pasteur, dans le laboratoire du regretté professeur Borrel, et ne tarda pas à être chargé, dans le cours de Microbiologie générale professé dans cet Institut, de l'enseignement de la Mycologie. Il prit le grade de docteur ès sciences en 1907, avec une thèse sur le ,,Rôle des bactéries dans le développement de certains Myxomycètes''.

Au moment de l'entrée de Pinoy à l'Institut Pasteur, la théorie phagocytaire de l'immunité, développée par Metchnikoff, était en plein triomphe, et l'étude des phénomènes de phagocytose était à l'ordre du jour. Il était intéressant de les rechercher chez des organismes tels que les Myxomycètes, qui se nourissent de bactéries, et Pinoy se consacra à l'étude de ce groupe. Les Myxomycètes sont des êtres très singuliers, dont on ne sait au juste s'ils sont des végétaux, voisins des champignons, ou des animaux se rattachant aux Protozoaires du groupe des Rhizopodes. Ils se présentent, à l'état végétatif, sous forme de masses protoplasmiques multinucléées ou plasmodes, qui peuvent atteindre de grandes dimensions et qui rampent à la surface de substratums tels que des branches pourries. Ils développent des fructifications généralement formées d'un pied que surmonte un sporange renfermant des spores. La spore germe en une zoospore ciliée qui, par perte de son flagelle, se transforme en une cellule rampante semblable à une amibe, la myxamibe. Les plasmodes se forment par fusion de nombreuses myxamibes et le cycle se trouve ainsi fermé. Les Myxomycètes sont associés à de nombreux microorganismes, bactéries et champignons. Pinoy a mis en évidence la grande importance que peut prendre l'association des bactéries dans la biologie de ces organismes. Il a réussi à obtenir des cultures pures mixtes où le plasmode du Myxomycète est associé à une seule espèce bactérienne. Dans ces conditions, il a constaté que les myxamibes ingèrent les bactéries et les digèrent dans leurs vacuoles, a l'aide d'une disatase agissant en milieu alcalin, neutre ou légèrement acide, voisine de l'amibio-diastase. Pour réaliser ses expériences, Pinoy ajoutait à des cultures de plasmodes, purifiées par passages successifs sur des milieux de culture peu favorables aux bactéries, diverses bactéries en culture pure. Le bacille tuberculeux n'est pas digéré par le Myxomycète. Les bacilles paratuberculeux, au contraire, permettent le développement. Le colibacille convient bien, et avec lui, le Myxomycète donne d'abondantes fructifications. Le B. typhique, au contraire, n'a pas permis le développement. Le pigment des bactéries colore souvent les plasmodes.

Enfin Pinoy a découvert, chez les Myxomycètes, le phénomène de la sexualité hétérothallique; il s'est comporté là en précurseur, puisque ce processus a été retrouvé par la suite dans d'autres groupes de Champignons (Basidiomycètes, Ascomycètes).

Les recherches de Pinoy sur les Myxobactéries ne sont pas moins remarquables. En culture pure, les Myxobactéries ne se distinguent en rien des bactéries les plus banales. Mais dans certaines conditions, elles édifient des appareils complexes qui, chez le *Chondromyces crocatus*, une des espèces les plus évoluées du groupe, sont formés d'un pied ramifié, portant à l'extrémité de ses rameaux des têtes sphériques sur lesquelles s'insèrent régulièrement des kystes ovoïdes. En dépit de leur forme compliquée, ces productions ont une structure très simple: elle sont constituées par des éléments bactériens disposés en alignements réguliers, enrobés dans une substance unissante de consistance dure et de couleur jaune orangé vif. Pinoy a montré que la condition *sine qua non* de l'apparition de ces appareils dits de fructification, est que le *Chondromyces* soit associé à une bactérie voisine du *Micrococcus luteus*. Si l'on ensemence sur le même milieu, simultanément, le *Chondromyces* et le *Micrococcus*, on constate que ce dernier est solubilisé par la Myxobactérie; puis les appareils de fructification s'édifient au niveau des plages bactériolysées. Le même résultat est obtenu, si l'on ajoute à une culture pure de *Chondromyces* un extrait chloroformique de *Micrococcus luteus*. Enfin, si l'on associe au *Chondromyces crocatus*, non plus le *M. luteus*, mais une autre bactérie (*B. fluorescens*, *B. pyocyaneus*), on obtient des formes anormales dont la plus intéressante, avec ses kystes disposés en chapelets, se rapproche beaucoup du *Chondromyces catenulatus*. On assiste là, sous l'influence d'une condition parfaitement déterminée, à la transformation d'une espèce en une autre. Découverte d'une portée considérable, capable peut-être d'apporter des clartés nouvelles dans le problème de l'Evolution, qui n'a pas eu en son temps le retentissement qu'elle méritait (Pinoy ignorant totalement l'art de se faire valoir), mais que l'avenir, sans nul doute, remettra à sa vraie place.

Dans le domaine des mycoses ou maladies humaines et animales provoquées par les champignons, Pinoy s'est comporté en pionnier. Il était bien préparé à cette tâche par sa triple éducation de mycologue, de médecin et d'anatomo-pathologiste. Il commença par apporter de l'ordre dans la question; au moment où il aborda ces recherches, de nombreuses mycoses avaient été décrites, mais souvent par des auteurs peu familiarisés avec la mycologie, d'où l'insuffisance de leurs descriptions botaniques. Dans un premier article (1903), Pinoy établit la classification des champignons pathogènes pour l'homme, les animaux et les plantes, d'après leurs caractères botaniques. Il aborda ensuite, parmi les nombreux types de mycoses, l'étude des mycétomes. A côté des mycétomes à grains toujours blancs ou rosés qui reconnaissent pour agent non un champignon, mais un Actinomycète (*Nocardia madurae* Vincent), existent une ou plusieurs variétés de mycétomes dans lesquelles les grains peuvent se présenter avec des teintes variables, blanche ou noire. Les champignons, jusqu'alors non cultivés, décrits dans ces formes, offrent un ensemble de caractères communs: ils se présentent dans les lésions sous l'aspect de tubes mycéliens cloisonnés et ramifiés, montrant sur leur trajet ou à leur extrémité des chlamydospores. Leur membrane est parfois brunâtre et le pigment qu'ils sécrètent peut diffuser autour d'eux. Avec le regretté CHARLES NICOLLE, PINOY étudia un pied de Madura à grains blancs appartenant à cette catégorie. A partir des grains, il obtint la culture pure du parasite, qui est une variété de l'*Aspergillus* (*Sterigmatocystis*) *nidulans* Eidam. Il s'agit bien du parasite, car dans les grains on peut rencontrer des hyphes fertiles portant des conidies. On devait donc admettre l'existence d'un mycétome aspergillaire. Les travaux ultérieurs ont montré l'importance de cette forme: M. BRUMPT a décrit un mycétome de la main à *Aspergillus bouffardi*. MM. BLANC et LANGERON ont décrit un mycétome de la main à *Sterigamocystis tunetana*. Avec M. MASSON, PINOY a décrit un mycétome aspergillaire du poumon chez l'âne.

Jusqu'alors toutes les tentatives faites pour reproduire expérimentalement les mycétomes du type "pied de Madura" avaient échoué. PINOY est parvenu à obtenir un mycétome à *Sterigmatocystis nidulans* chez le pigeon. Le champignon a été entraîné à se développer dans de mauvaises conditions, à l'abri de l'air, en bouillon sucré, sous une couche d'huile de vaseline. La culture a été inoculée dans la patte d'un pigeon et, au bout d'un mois, sur la peau de celle-ci, on voyait pointer des grains noirs de la grosseur d'un grain de chènevis. Ces grains sont constitués par des filaments fortement pigmentés. Il n'est pas sans intérêt de constater qu'un champignon isolé d'un cas de mycétome blanc chez l'homme peut donner un grain noir typique chez le pigeon.

Toujours avec CHARLES NICOLLE (1908), PINOY a étudié un mycétome à grains noirs observé dans le Sud-Tunisien, à Tozeur. Le champignon isolé de ces grains ne donne pas de formes parfaites. Il a été rapporté, sous le nom de *Madurella Tozeuri*, au genre *Madurella* Brumpt, em. Pinoy, dont PINOY a publié par la suite une révision (1912). Dans le cas en question, la structure des grains était assez spéciale: les filaments mycéliens suivaient le plus souvent les vaisseaux sanguins et les ramifications veanient former des boucles autour des capillaires. Les éléments (leucocytes mononucléaires, parfois cellules géantes) inclus dans la boucle noircissent d'abord en se colorant par le pigment du champignon et finissent par se transformer en une masse granuleuse, puis vitreuse, amorphe. Un grain peut être ainsi formé uniquement par une boucle mycélienne, renfermant une masse plus ou moins amorphe pigmentée. Le pigment du champignon est dû à la tyrosinase.

Nous avons signalé plus haut que, dans le mycétome à *Sterigmatocystis nidulans*, le champignon peut donner des formes conidiennes aberrantes dans le tissu qui entoure le grain. Les champignons pathogènes sont donc capables de fructifier à l'intérieur des tissus, chez l'homme, contrairement à l'opinion couramment admise à l'époque. A la liste des champignons aptes à fructifier au sein des tissus humains s'ajoute le *Sporotrichum beurmanni*, agent de la sporotrichose; Pinoy, en effet, dans un cas de sporotrichose généralisée, a observé des formes de fructification très nettes du parasite à l'intérieur des capillaires (1911).

D'une teigne exclusivement cutanée du singe, PINOY a isolé un champignon qui offre un intérêt considérable du point de vue systématique (1931). Par ses cultures, il se rapproche d'*Epidermophyton inguinale*, bien caractérisé par ses fuseaux. Les hyphes sporifères sont celles d'un *Trichophyton* ,,ecto-endothrix'', envahissant le poil à l'extérieur et à l'intérieur. Les fuseaux sont ordinairement à 4 ou 6 loges, séparées par des cloisons incomplètes. Le plus souvent, le contenu protoplasmique de chaque loge s'isole par une membrane propre assez épaisse de la paroi du sac et les spores ainsi formées sont expulsées soit par l'extrémité supérieure du fuseau quand il est encore fixé au filament, soit indifféremment par l'une ou l'autre de ses extrémités lorsqu'il est détaché et tombe sur le milieu de culture. L'évolution de ces spores au point de vue cytologique est très intéressante. Au début de la formation du fuseau, elles sont binucléées, puis les deux noyaux se fusionnent. Avant la germination ou au moment de la germination, le noyau de fusion se divise en deux, puis en quatre noyaux et une troisième bipartition porte à huit le nombre des noyaux. Ces noyaux passent dans le bourgeon qui s'allonge et se coupe en autant de cellules qu'il y a de noyaux; chaque article émet un petit bourgeon, dans lequel passe tout son contenu, protoplasme et noyau. Cette évolution nucléaire rappelle celle que l'on rencontre dans le cycle des Basidiomycètes parasites des plantes, Urédinées et Ustilaginées. Si bien que toutes les formes de fuseaux qui existent chez les champignons des teignes n'ont vraisemblablement pas la même valeur et si, comme il est probable, ces champignons sont des êtres dégradés par le parasitisme, il est possible que certains aient pour souche des Ascomycètes (*Gymnoascus, Aspergillus, Penicillium*), d'autres des Basidiomycètes. Cette découverte, qui repose sur ces constations cytologiques d'une admirable précision, est d'une extrême importance, car jusqu'alors on ne connaissait pas de Basidiomycètes parasites de l'homme et des animaux.

Pinoy est intervenu aussi dans une question qui a soulevé en son temps des controverses passionnées, celles des mycoses de la rate. Dans certaines splénomégalies existent des nodules dits de Gandy-Gamna, dans lesquels on trouve des fibres que Pinoy, avec Langeron et Da Fonseca, considère comme des fibres conjonctives dégénérées. Mais on y trouve aussi des productions ressemblant à de grosses chlamydospores et des têtes renflées entourées de petites sphères ressemblant à des spores; ces formes ont pu être reproduites expérimentalement chez les animaux par inoculation de têtes aspergillaires ou de chlamydospores. On voit aussi, dans les nodules de Gandy-Gamna, de fins filaments, parfois ramifiés, que Pinoy considère comme appartenant à des Champignons. Les nodules de Gandy-Gamna ont fourni à Pinoy, par ensemencement, des cultures d'un *Aspergillus* qu'il a placé auprès du *versicolor* dans le stirpe du *Sterigmotocystis nidulans*. Y-a-t-il mycose da le rate ou le champignon n'est-il qu'un agent d'infection secondaire? Ce point, conclut Pinoy, sera tranché seulement quand on sera parvenu à reproduire expérimentalement le nodule sidérosique.

Parmi les espèces nouvelles de champignons pathogènes décrites par Pinoy, signalons *Acladium Castellanii* Pinoy, 1916, isolé par A. Castellani d'ulcères cliniquement très caractéristiques, et *Enantiothamnus Braulti* Pinoy 1911, isolé par M. Brault d'une tumeur chez un Arabe.

Pinoy fut, parmi les microbiologistes, l'un des premiers à détacher les Actinomycètes des champignons pour les rattacher aux bactéries, opinion qui est aujourd'hui devenue classique. Parmi les organismes de ce groupe, il a étudié le *Cohnistreptothrix* (*Actinobacterium*) *Foersteri*, Actinomycète anaérobie trouvé dans les concrétions des voies lacrymales et très voisin, par ses caractères de culture et sa morphologie, de *Cohnistreptothrix* (*Actinobacterium*)*Israeli*, qui est le parasite le plus fréquent de l'actinomycose. Avec Ravaut ,il a découvert un nouvel Actinomycète parasite, *Cohnistreptothrix Thibiergei*, qui avait produit chez l'homme ue actinomycose généralisée à forme gommeuse simulant la sporotrichose. Les lésions renfermaient des grains avec couronne de massues.

Enfin, Pinoy a décrit, avec Ravaut, une actionobacillose à forme méningée, due à l'*Actinobacillus Lignieresi*, et survenue à Paris chez un Argentin (1911). Il a, d'autre part, observé à Paris plusieurs cas d'actinobacillose du bœuf et a pu obtenir des formes massuées en grand nombre sur les milieux peptonés, glucosés à 2 %, additionnés d'un tiers de sérum de bœuf.

Pendant la guerre de 1914, PINOY travailla activement pour la défense nationale et imagina un procédé de lipovaccination contre les fièvres typhoïdes, qui, grâce à la lenteur de la diffusion du vaccin en suspension dans un excipient gras, permettait de réduire le nombre des inoculations. En 1917, bien que dégagé, en raison de son état de santé, de toute obligation militaire, il contracta un engagement volontaire et fut affecté au Maroc, où il reçut la croix de la Légion d'Honneur à titre militaire. Il devait rester fixé en Afrique du Nord. En 1922, il fut nommé Maître de Conférences, puis professeur sans chaire de Botanique à la Faculté des Sciences d'Alger. En 1925, il devint professeur de Parasitologie et de Microbiologie à la Faculté de Médecine de la même ville. Les obligations professionnelles et l'âge ne ralentirent en rien son activité; récemment, avec M. MARCHAL, il élucidait, à l'aide de techniques de micromanipulation, le cycle évolutif d'un très curieux protiste, l'*Endococcus moriformis* (1943).

Par son œuvre si variée et si originale, PINOY, malgré son extrême modestie, s'était acquis une juste notoriété. Il fut deux fois lauréat de l'Institut. De 1914 à 1917, il présida la Société Mycologique de France. Membre correspondant de l'Académie de Médecine, il fut élu, en 1946, correspondant de l'Académie des Sciences pour la Section de Botanique.

Tous ceux d'entre nous qui ont connu ERNEST PINOY garderont le souvenir de l'aménité de son caractère, de son inaltérable bonne humeur, de son large sourire, de son esprit incisif et du regard pénétrant de son œil clair, apte à percer les mystères qui restent cachés aux observateurs ordinaires. Il était doué d'un tempérament d'artiste: organiste et compositeur de talent, il avait écrit des fugues et s'était lié avec le grand maître LOUIS VIERNE, qui lui confiait parfois le grand orgue de Notre-Dame de Paris, instrument admirable dont il était le titulaire.

Atteint par la retraite, PINOY avait quitté Alger et avait décidé de reprendre sa place dans le laboratoire de Mycologie de l'Institut Pasteur, qu'il avait fondé et où j'ai l'honneur de lui succéder. C'est au laboratoire même, où il venait à peine de reparaître, qu'il ressentit les premières atteintes du mal qui devait nous l'enlever si brusquement. Avec lui disparaît un spécialiste éminent de la Mycologie Médicale, des Myxomycètes et des Myxobactéries, un expérimentateur ingénieux et habile et, surtout, un penseur aux idées larges, dont les découvertes de grande portée font honneur à la Biologie contemporaine.

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Enzymatic grouping of Bacteria

by

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Summary. — An enzymatic tripartition of bacteria is suggested, on the basis of their glycolytic and proteolytic metabolisms, drawing the attention about its advantages, for what concerns both their scientific study and their functional employment in the zymotechnical and medical fields after up-to-date directions.

The subject I intend to deal with is very wide and difficult; I wished to discuss it thoroughly, but, because of the present typographical scarcities, I must carry it out ,,*per summa capita*'', enlighting nevertheless the importance deserved by the bacterial enzymology either of the theoretical and the applied science, or the zymotechnic and the medical matters, since I am led to this by my intuition and by the multilateral and complex formation I enjoyed, in the same time biological and chemical, medical and zymotechnical, both in theory and practice, which allowed me to reach an unitarian view of the microbiologic phenomena.

I am talking of *grouping* and not of classification, because the latter requires the use of systematic taxonomic rules which undergo continuous changes, and which may never be fixed as far as science could not reach the full knowledge of the true being, the intimate nature of bacteria, their behaviour as beings living in accordance with the economy of the whole natural world; as long as to-day, their behaviour has been studied almost exclusively by the standpoint of their relations to the health and economy of the man. The numerous bacteriological classifications presently on hand are therefore all defective, and moreover they show large differences, since the basis upon which they are built up is, from time to time, either morphologic or physilogic, either biologic or chemical, either medical or zymotechnical.

Thus, according to the different taxonomic criterions taken as a basis, some AA. distinguish into the Coccaceae family nine genera, others six, others three and others only two genera (*Streptococcus* and *Staphylococcus*); in the genus *Streptococcus* some include a number of saprophytic species and only a parasytic one (*Str. pyogenes*), others, on the contrary, put in a number of parasytic species and only a saprophytic one (*Str. faecalis-lactis*); in the family of Bacteriaceae some see twenty-four genera, others a far lesser number, identifying for instance the genus *Eberthella* with the *Salmonella*, the genus *Escherichia* with the *Aerobacter*, and so on; some aknowledge a unique species of lactobacilli, whilst other list some thirty-five species. And we don't bother, here, with terminology, which undergoes everlasting and puzzling changes! It may be sufficient to compare the one with the others the classifications by Migula, Chester, Buchanan, Winslow, Topley and Wilson, Lehmann-Neumann and the six Bergey's editions, and even the two Orla-Jensen classifications of the lactic acid bacteria (1919 and 1943).[1)]

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1) It may be interesting to record the following numbers of scientific names that appear in the successive editions of the Bergey Manual: 1923, 1st ed., 1643; 1925, 2nd, ed., 1,816; 3nd ed., 2,293; 1934, 4th ed., 2,851; 1939, 5th ed., 6,405; 1948, 6th ed., 12,512. And similarly the genera and the keys are stil changed.

In front of so strong differences and so high a confusion, it is clear the time has not yet come for the definitive settling of rules concerning either the terminology or the net distinction between species and species, and between genus and genus.

On the contrary the enzymatic grouping does not bother either with the morphology or the terminology of bacteria; each group can include microorganisms with the most different morphologic characters and whatever their names, even if the latters do not respond to the taxonomic rules and appears as synonyms or less defined names.

The enzymatic grouping is built upon the bacterial metabolism, which presently is very closely bound to enzymology, and it can therefore be divided into as many chapters as are the metabolic characters one puts into consideration.

These groups, obviously, may vary both in number and importance, according to the medical or zymotechnical point spotlighted in the particular moment, but the method allows anyway an impartial grouping, suffering no limits, since it can withstand any kind of bacteriology, whatever the mind, the culture and the direction of the students; moreover it deserves also a theoretical and practical value in the same time, and it puts the possibility of studying and making use of the bacterial activity into the zymotechnical as well as into the medical processes, and the easiness of enlighting the relations linking the zymotechnical and medical bacteriologies.

Starting from the principle that all the activities of microorganisms are of enzymatic nature, each of them could be taken as a basis for enzymatic groupings [1]): the grouping, however, that seems to me the most important, general and comprehensive, being the one interested into the two basilar metabolic activities: *glycolisis* and *proteolysis* under their large significance [2]).

Such a grouping must be based upon the microbial behaviour into *media containing the convenient glycides and proteins*, resembling the ones we find commonly both in the zymotechnical and the medical fields. Such a medium is given by the *natural milk*, with no substances added, because it can be regarded as a complete natural nutritive medium, comparable to the *blood*, since both contain all the necessary organic substances in their native and complex form, as well as biogenetic mineral elements, vitamins and other growth-promoting and enzymatic principles. As a matter of fact milk appears, in the same manner as the *blood serum*, fit for the growth of the generality of microorganisms, there included the most claiming pathogenic ones. The aforesaid grouping, indeed, depends, for its origin and development, upon the researches I made about the behaviour of bacteria into milk.

1 — *Enzymatic tripartition of bacteria.* — I started from my discovery (1892—94) that, in addition to the bacteria curdling the milk by acidification, that is by glycolysis, and the bacteria curdling by rennet, that is by caseolysis, there are some bacteria curdling it simultaneously by the two ways; after this observation I suggested a division of the milk bacteria into three groups: 1) plain lactose bacteria, forming acid solid and integer clot and unable to dissolve it again (*common lactic acid bacteria*); 2) plain casein bacteria, giving either neutral or alkaline soft clots and able to dissolve them again always in neutral or

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[1]) Frei, for instance, grouped bacteria into six classes, after their respiratory enzymatic systems (Zentr. Bakt. I Or. 1935).

[2]) Under the narrowest significance, both glycolysis and proteolysis would be restricted to the hydrolytic processes, resulting on one side in the by-products of saccharides by action of carbohydrases, and on the other side in the aminoacids, by action of proteases.

alkaline reaction (*common peptonizing bacteria*); 3) double lactose and casein bacteria, giving an acid clot and dissolving it again in acid reaction (*acid-rennet bacteria* or, rather better, *lactic acidoproteolytic bacteria* (since the rennic activity is just a proteolytic action, the chymase being a proteinase). These bacteria of the third group give first an ordinary uniform curd like the common lactic acid bacteria, but after two days the curd begins to dissolve and it assumes various *characteristic* shapes; sometimes digestion occurs from one side only and the curd is forced against the wall of the test-tube, to which it remains attached; sometimes digestion occurs from all sides and the curd assumes the shape of a pencil floating in the middle of the whey; all these curds may show transverse or longitudinal clefts filled with whey (but lacking gas) which may be regarded as lines of digestion; the whey is generally clear, colourless or pale yellow, with an increasing acidity; digestion is rarely complete (see fig. 1).

The observations I put forth resulted in the unsettling of two then commanding opinions: 1) that the glycolysis and the proteolysis were antithetic processes, because of which fact they could not be both present; 2) that bacteria could perform proteolytic action only in alkaline or neutral medium, by means of common tryptases.

Obviously, if one does not consider the acidoproteolytic organisms, and it deals only with the plain bacteria, the aforesaid opinions still maintain their value in various bacteriological data. It is true, for instance, that in microbial cultures the sugars carry out a sheltering activity on albuminoids; I too have given the demonstration of this fact as far as in 1893[1]) dealing with cultures of *Vibrio cholera*, which organism does not form indole when the peptone-broth contains also glucose; this observation has been afterwards supported by other AA. For what concerns both the organism of cholera and of *B. coli*, and various other microorganisms [2]) into different culture media, and again in the recent years (1938) by WOHLFEIL, who used blood-serum, and by SCHOLLMEYER (Zentr. Bact. I, 1938) who could demonstrate the sheltering action of sugars on the colloidalysis also in presence of substance hindering the action of the colloidolytic enzyme, thus recording the addition of the two antiprotease effects. It is also true that glycolytic microorganisms, such as the coli and the diphtheric, can attack the proteins only if the medium lacks of sugar, making use of the former substances as energy sources, instead of the latter ones; and that the common proteolytic germs dissolve the ordinary gelatine, but not the sugared one, while the acido-proteolytic germs dissolve also the latter, as I could demonstrate back in 1902, and sometimes, moreover, they refuse to attack the common gelatine.

No surprise therefore can give the fact that, because of its absolute novelty, or better the true precociousness of the discovery of the acidoprotelytic organisms, related to the time (we were then at the first beginnings of enzymology), my researches and my suggestions remained neglected for a long time, also for the reason that the first acidoproteolytic organisms I pointed out, though isolated out of the market-milk, belonged to common saprophytic species (*B. prodigiosum, B. indicum, Proteus mirabilis, Ascobacillus cireus*). Only ten years later, when I could consecutively demonstrate the existence of acidoproteolytic microorganisms interesting the dairy, the agriculture and the medical science, and not only through their saprophytic life (into milk, dairy products, fodder and soil) but even through their parasytic or semiparasytic life (into udders, stomach, intestine), and moreover also some belonging to the pathogenic bacteria (*Staphylococcus pyogenes*) the new microbial group attracted the attention of bacteriologists, both in biology and chemistry, and in the medical, agricultural and industrial fields.

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[1]) Central. Blatt. Bakt., 1893, XIII, p. 700.

[2]) For instance the pigment production by my *B. lactis niger* which is dependent upon aminoacids, is hindered by the presence of glucose, since the latter shelters the proteic substances (CLARCK and SMITH, Journ. Bact. 1939).

It is quite natural, however, that here one is not dealing with an absolute distinction (where are, please, the absolute distinctions in biology?). One must however concede that the whole bacterial stock, since it feeds upon the organic ternary and quaternary substances, is obliged to maintain both the carbohydrasic and proteasic properties; certainly also, it is not correct to think that bacteria are thoroughly exclusive in their nutritive needs; in regard to this point I have observed, back in 1892, contrary to the common belief, that the production of enzymes may be undependent upon the nutritive needs, since I demonstrated that *B. prodigiosum* put forth chymases *as a customary habit*, that is also into media lacking of casein, observation from which I started for my division of enzymes between *habitual* (customary) and *adaptive* ones (1940) instead of the division in constitutive and adaptive.

But besides their nutritive activity, bacteria have to provide for their energetic activities, which build up, just so, their fermentatic processes. One can however subtilise as far as the extreme limits, but when bound to the observation that, "*coeteris paribus*", out of three bacteria sown into *natural milk* (that is milk free from any modification or addition) one makes it acid and curdled, and it is not able to dissolve again the clot, a second one peptonizes it without acidification, on the contrary alkalinizing it, and the third acidifies and petonizes it in the same time, one is led to the obliged conclusion that the three microorganisms maintain different enzymatic properties.

As a matter of fact, *the differences into the enzymatic production by bacteria* may be dependent upon three factors: *quantity*, *quality* and *characters* of their enzymes.

For what concerns the *quantity* of enzymes, one can plainly concede that the first microorganism is richer in glycolytic than in proteolytic enzymes, that the second one is, on the contrary, richer in the latter ones, and that the third contains about the same amount of the two types of enzymes. For what concerns the *quality* of enzymes, the acidoproteolytic, while were the first ones to warn that the microbial proteases are different from the tryptases, to-day are considered as producers also of peculiar proteases, acting under strong acid reaction (GORBACH acidoproteases "Enzymologia" 1935—37). But, moreover, it is necessary to take into consideration also the *characters* of enzymes which may be different at least by three points; their *complexity*, *sensibility* and *liberation* (delivrance).

For what concerns the *enzymatic complexity*, the development of the enzymological studies resulted in the knowledge and the isolation of a number of carbohydrases and proteases, so that now the students deal rather with enzymatic systems, than with single enzymes, and we have reached the enzymatic dissociation (ALBERS). For what concerns the *sensibility*, which is dependent upon the higher or lower activation-degree under which the enzymes are produced, we know that the enzymes behave differently against the stimulation and hindering factors, and that their action needs, at a larger or narrower degree, the help of some auxiliary factors, known as co-enzymes.

For what concerns their *liberation*, that is the easiness with which the enzymes are discharged, it is sufficient to remember the differences between the endo- and exto-enzymes, and between the desmo- and lyo-enzymes (WILLSTÄTTER), and moreover the different speed under which the cellular autolysis is obtained in different bacteria. Although the true reasons and conditions starting the autolysis are at yet unknown, it is quite interesting the parallelism between its speed and the proteolytic potency into some bacteria (DERNBY); for instance in *Proteus* and in the lactic acidoproteolytic cocci autolysis is swift and proteolysis sharp; in *B. coli* and the common milk bacteria the former is slow and the latter weak.

That said, and maintaining always under consideration the inevitable biologic limitations, the enzymatic grouping I suggested does not stand limited only to the bacteriology of milk, but it can to-day cover other microbiological fields, in general line, both in zymotechnics and medicine; it deals not only with lactose and casein, but with glycides and protides in general.

Bacteria may therefore be divided into three enzymatic groups, according to their primary activity: 1°) bacteria of glycides, viz. *glycolytes*; 2°) bacteria of protides, viz. *proteolytes*; 3°) simultaneous double bacteria of both the said substances, viz. *glycoproteolytes*, which I preferably would name *acid-proteolytes*, in order not to mistake them whith the microorganisms which are really able to utilise both glycides and protides, but never simultaneously, and, in any case, *never under a strong acid reaction*, which latter fact, on the contrary, builds up the precise character of the true lactic acidoproteolytic bacteria, acting at a pH = 3.5—4 (GORINI, GRASSMANN and SCHLEICH, 1933 — Z. physiol. Chemie); these bacteria therefore performed a sharp change into our knowledge concerning the *fermentation of organic nitrogenous substances*, for what regards the critic acidity for their disintegration and, as a consequence, for their preservation.

As a matter of fact, the common lactic acid bacteria are able to dissolve the casein only when they are cultured in milk to which have been added some substances (calcium carbonate and similar ones) which neutralize the acidity carried out by the glycolysis; on the other hand we know some common peptonizing bacteria (*B. subtilis*, *B. pyocyaneum*, *Sarcina lutea* and others) which, while degrading the casein, degrade at the same time also lactose, but never under acid reaction, because they, either do not produce acidity or produce it at such a small degree that the same is neutralized by the buffer action of the medium or else by the simultaneous production of basicity (WEDUM, 1939). It is therefore useful to separate a proteic *putrefactive* fermentation, carried out by the alkalinoproteolytes, from a *digestive* proteic fermentation performed by acidoproteolytes.

It is also useful to warn that the acidoproteolytes, also named double ferments, are not to be mistaken with the so-called "mixed ferments" described by TISSIER and MARTELLY (Annales Pasteur, 1902), which, though utilizing glycides and protides, contain in the same group both acidoproteolytes (f.i. *Staphyl. pyogenes*) and alkalinoproteolytes (f.i. *B. pyocyaneum*). It is very likely that the said authors did not know the researches I published ten years before [1]).

Following the aforesaid ideas, *to-day also the pathogenic germs are divided into the following three groups, according to their primary enzymatic activity* in blood serum, which is a substrate equivalent to the milk: 1°) germs showing carbohydrasic activity (corresponding to glycolytes); 2°) germs demonstrating proteasic activity (corresponding to proteolytes); 3°) germs possessing enzymes either active or easily activable belonging to both the said groups (corresponding

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[1]) Still to-day, reviewing the existing literature, one is led to the conclusion that the possibility of a microbial fermentation of the nitrogenous substances under strong acid reaction, resembling the one carried out by the acidoproteolytes, is not sufficiently spread.

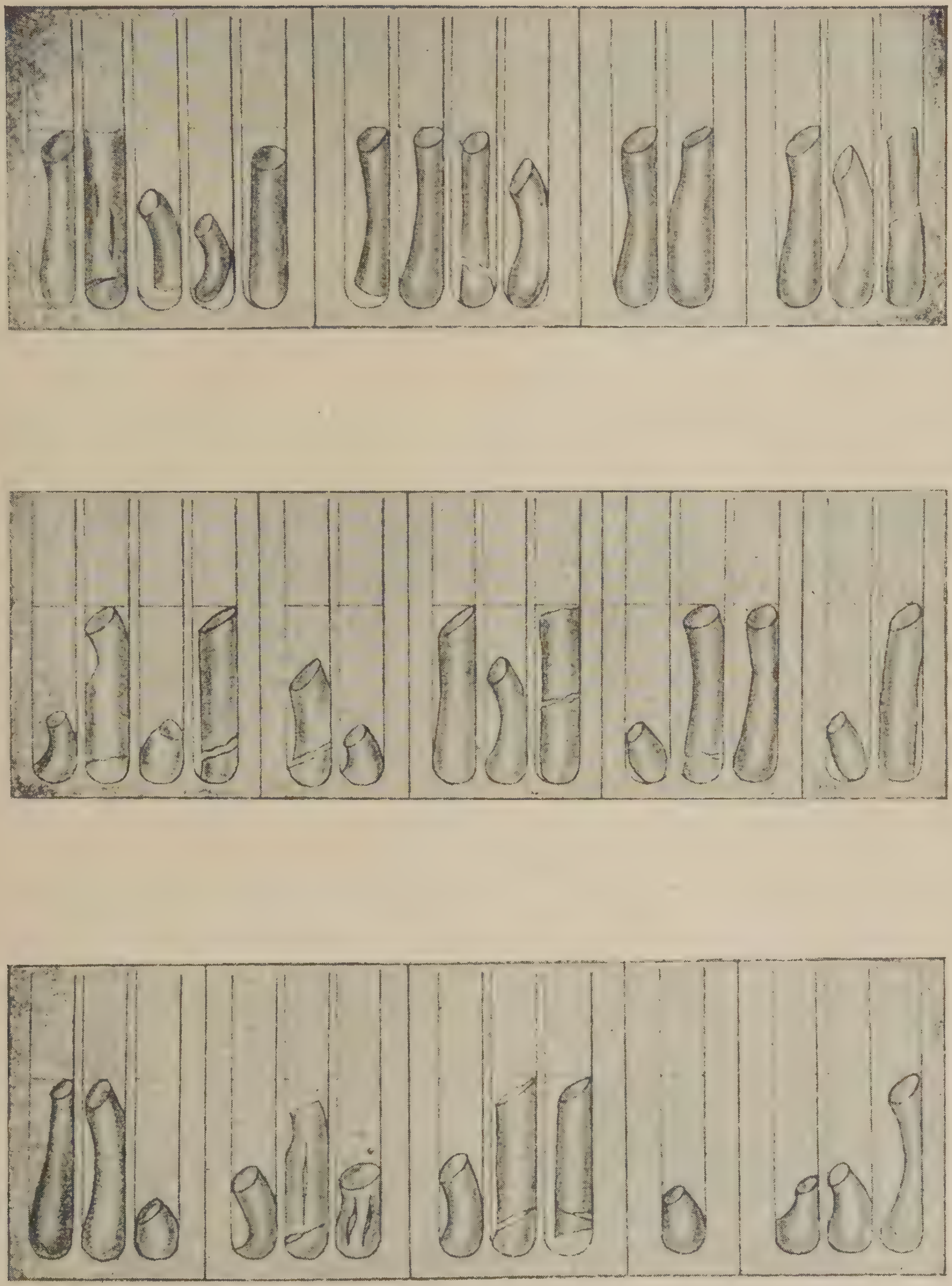

Fig. 1. Characteristic milkcultures of various stocks of lactic acidoproteolytic uddercocci (P. Rencodis.)

apt, but, to the development and the normal activity of the milk common lactic acido bacteria and to the lactic acidoproteolytes, which should be aseptically and recently squeezed (so that it would not be previously altered by possible contaminants), and blandly sterilized at its white colour (so as to interfere the least with its intimate structure, by means of heat). All these conditions, which may denaturate, in a deep grade too, though obscurely, the proteic and sugar contents of milk, are sufficient to explain the results often strongly different or opposite obtained during the researches about the proteasic and glycolytic actions of some germs in milk, leaving apart also all the considerations on the use of milks to which are added neutralizing or other different substances, or of milks in any way modified. As a general rule, the drastically sterilized milk favours the glycolysis and it is unfit for the proteolysis; there exist, however, some exceptions.

It must be noted, however, that the different proteolytic behaviour of bacteria in the milk more or less deeply sterilized by means of heat, finds its reason in their different proteasic production, due to the fact that, according to its lesser or higher rate of *proteinases* or of *lower proteases* (peptidases), bacteria can make use of the native casein of the crude milk (*proteinolytes*) or rather of the casein already hydrolized by heat (*pepotlytes*).

It is unnecessary to note that analogous conditions, because of the sharp sensibility of bacteria against the external factors, make very difficult the study under constant cultural conditions of the microbial enzymatic activity against the generality of protides and glycides. In this connection it is convenient to observe that the up-to-date enzymology must explain why the fermantative power of germs on glycides appears so variable in dependance of the aeration, the temperature, the nature of the medium, and the isolation time of the microorganism from its natural *habitat*, so that the same fermentative power cannot still be considered as a constant character of a given species or variety or strain; to-day, indeed the glycolytic reactions, whose diagnostic value appeared so reliable, are adopted and thought of as auxiliary differential criteria, instead of as primary ones [1]).

c) *Saprophytic and parasytic life* — On the subject of the proteolytic and glyolytic variations demonstrated by the germs extracted from their original ,,*habitat*'', I could observe that the living mood being more saprophytic than parasytic, or semiparasytic, means a very complex ambiental condition [2]). With regard to this point I detected a bright difference in some species of streptococci, staphylococci, *B. coli*, whose saprophytic strains show a high glycolytic potency and a weak proteolytic power, whilst the parasytic strains demonstrate an opposite behaviour; this fact has been confirmed also by recent researches of other authors (POCHON, C. R. Ac. Sc., 1939; MADISON, Proc. Soc. Exper. Biol. N. Y., 1935) and it explains the wide distribution of the rennet-curdling and (even feebly acidoproteolytic) properties I detected in the pathogenic germs (1923) above all among the ones freshly isolated from the living body (*B. typhi*, 1926).

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[1]) The same ORLA-JENSEN, who divided the lactic acid bacteria on the basis of the series of attacked glycidesi was led to the conclusion that they appear, for what concerns this character, often whimsical and "lunatic". My theory of *genotypicity of microbial enzymes*, however, gives new value to these criteria, when it is maintained in consideration with the oecologial history of bacteria (see later).

[2]) I remember that the first observations about the relations between the microbial enzymes and the pathologic processes are referred to the sharper proteasic activity of the virulent ones; they have been detected at first for what concerns the degradation of peptones (ROSENTHAL and PATAY, Zbl. Bakt. Orig., 1914), and later for what concerns the degradation of casein (GORINI, 1924—1927), the horse serum (WEISSFEILER, Zbl. Bakt. Or. 1928 and SCHERN, Berl. tierarz. Wschr., 1936) and for other substances. It was used in the plasmocoagulase (which is similar to chymase) test for the diagnosis of the pyogenic and enterotoxic staphylococci and of the virulent *B. coli* (CRUICKSHANK, J. Path. Bact., 1937), (CHAPMAN, J. Bact., 1937), (LUOKING, Zbl. Bakt. Ref., 1937), (COWAN, J. Path. Bakt., 1938).

For all these reasons, the same bacterium may belong to the one or the other of the groups, according to the research-field; the ,,milky'' division cannot correspond to the medical one (bloody division) or to the others. Here is an example: before I published my researches, *B. prodigiosum*, according to its dissolving action upon gelatin was considered a plain alkalinoproteolyte, also *B. pyocyaneum* and *B. fluorescens*; on the contrary I demonstrated that in the milk, as well as in the lactosed gelatine, *B. prodigiosum* behaves as an acidoproteolyte, while both *pyocyaneum* and *fluorescens* still act as alkalinoproteolytes; recently the enzymologic control allowed us to detect precisely an essential difference between the proteasic systems of these germs (Gorbach, Enzymologia, 1935—37; Moycho, C. R. Ac. Sc., 1936; Maschmann, 1943).

The same behaviour of *B. prodigiosum* can be observed near the pyogenic staphylococci, which in the milk appear acidoproteolytic (Penso e Rochais), while in the broth are alkalinizing; on the contrary the same conduct of *B. pyocyaneum* and *B. fluorescens* can be observed also in *B. alcaligenes*, *B. enteritides*, *B. foetidus lactis* and *B. parathyphi B.*, which too in milk are alkalinoproteolytic.

III — *Variability of the enzymatic activities due to individual divergence.*

But there exists another factor maintaining an essential value with regard to the enzymatic variations: the factor of the *microbial individuality*. Still on the basis of the peculiar behaviour of acidoproteolytes in milk, I could enlighten the phenomenon of the *microbial dissociation of biochemical order*, in the same year in which Arkwright attracted the attention of the students upon the phenomenon of the morphological dissociation (1921) [1]. And here is how it has been done.

a) *Spontaneous variations.* — During the fifty years through which I dealt with a rich collection of Acidoproteolytes, by which I systematically got subcultures, I observed the appearance of *sudden spontaneous variations* in their double saccharolytic and proteolytic activity, so that sometimes some subcultures curdled without peptonization, or else they peptonized without curdling; and this took place independently on the influence either of external factors, or of ,,*modus operandi*''; setting, however, a *series of parallel subcultures* by the same mother culture, although monocytogenetic, I showed that the variation does not appear in all the subcultures, but only in a few of them; I was led to the conclusion that here one is not dealing with a total and global transformation of a given culture, but with a phenomenon affecting only parts of it. In order, therefore, to explain this fact, I was led to the idea of an individual enzymatic difference among the cells of the same acidoproteolytic culture, so that each culture must contain three enzymatic types of cells: the first mainly glaxcolytic; the second mainly proteolytic and the third endowed with balanced enzymatic activities (*perfect* or *exact* forms); through the said explanation, the observed variations lose the meaning of abnormal occurrences, entering the range of the normal phenomena, only depending upon the fortuitous chance of seeds made up either mainly or almost exclusively by cells belonging to a unique type; the variation can be transmitted by inheritance, however, as far as the mutant variety, always containing divergent cells, can still by chance, give origin either to a culture returning to the type of the originary variety (retrovariation), or to another divergent variety. In this case, therefore, one is not dealing with a persistent mutation (after De Vries), but on the contrary with fluctuative or alternate mutations.

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[1] The peculiar fitness of the milk, as a cultural medium, to the carrying out of the bacterial dissociation has been afterwards detected by other Authors (Nungester W. J. — J. Infect. Dis., **44**, 73, 1929. Hoogerheide, Jl. Bact. 1939).

Nor is one here dealing with a weakening or a degradation of the culture, in the first place because not all the cells are modified, in the second place because the modification does not affect both the enzymatic activities, but only the one or the other of them; (uncoupling of the activities) the variations are, instead, dependant on the cellular enzymatic differences; it is quite sufficient to get several subcultures by the same strain, in order to obtain again a normal culture or an *exact* or *perfect* one. [1])

This explanation of mine has been strengthened by the observation that the appearance of such modifications is favoured by the customary use of small inseminations, taken from a unique point of the mother culture; evidently the different cellular enzymatic types, seem irregularly spread in the zones of the culture and in accordance with the age of the same cells. We know very well, to-day, the importance maintained by the phase of the microbial growth in relation to the production of enzymes and in the metabolic activity as a whole (Winslow, Journ. Bact., 1935; Wooldridge, Bioch. J., 1936; Clifton, Hegarty, Journ. Bact., 1939); so that in the researches about enzymes it is necessary to note precisely the incubatory time of the cultures. In order to avoid such variations and dissociations, I suggested the techniques of *preparing a great deal of parallel inseminations, making use of abundant material, taken away from all the zones of a well developed mother culture*; I am glad to see that these cases, to-day, suggested also for what concerns the culture of pathogenic germs, which are regarded as showing individual differences as well, have a sound value in the clearing of the variations affecting the bacterial pathogenicity and toxicity, which now are ascribed to fortuitous discontinuous varieties, or to selections belonging to the normal life cycle of the germs, rather than to deviations from normalcy. As a matter of fact, now the *spontaneous variations remain contained within the pattern of the physiologic phenomena.*

Recently, moreover, the necessity of ample inseminations and generally of large quantities of bacterial substance for all the enzymologic studies, has been admitted also by other authors (Wohlfeil and Schollmeyer, 1938). And strongly probativa are, in this connection, the recent researches about the dissociation of common lactic acid bacteria, after which the classic *Str. lactis* appears as a group of types, each endowed with different carbohydrasic properties; this fact does not seem attributable to a mixture of strains, but, on the contrary, to individual cellular variants, because of the monocytogenetic derivation of the mother culture (Vas and Csiszak, Le Lait, 1939; Sherman, Journ. Dairy Sc., 1937). These precautions, however, are still not sufficient; it is moreover necessary to avoid the aforesaid cultural conditions, often obscure, which can carry out the excitement of the normal individual differences.

As a conclusion: as well for the influence of the life conditions differently acting upon the different enzymatic activities, as, for the influence of the individual variations, the same germ can appear, also in the same substrate, sometimes as a glycolyte, sometimes as a protelyte or an acidoproteolyte. A correct diagnosis must be based upon the said precautions.

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[1]) Also the spontaneous and sudden appearance, within a microbial species, of strains differently requiring particular growth factors, can be explained with the principle of the individual divergences (E. Moller, Berl. Chem. Gesells., 75, 9, 1108, 1942; F. Kögl and al., Zeits. physiol. Chemie, 269, 97, 1941).

A good method to avoid the influence of the individual differences, building up at the same time, at the best degree, the various most favourable medium conditions, is, for instance, offered by my method of ,,milk on agar-culture'' (1932) after which, rather than directly inseminating the germs into the milk, as it is the common practice, one pours the milk, suitably sterilized, upon an agar-streak-culture already well developed, completely covering it. The cultures must be maintained in suitable conditions, above all with regard to temperature; as a general rule the mean optimal temperature ranges between 25° and 30° C., since at higher temperatures, which, as we would see, stimulate mainly the glycolysis, the latter can be carried out too swiftly and deeply, so as to reach too precociously the degree of a ,,stop acidity'', which latter condition interrupts any other bacterial activity.

By my method one can observe the curdling activity into germs which seem commonly deprived of it, as B. typhi, *Str. equi* a number of *Streptococcus* and *Staphylococcus. Micrococ. lysodickticus* a number of Saccharomyceten and Actinomyceten etc. [1]

b) *Adaptive variants* — We must moreover remember that some germs, in order to perform their enzymatic activities, need the presence of exciting substances, among which I have named peptons, the blood, and vitamins; this is a question related to the one remembered above, concerning the activators and inhibitors acting on the enzymes, and which may be also applied to their diagnosis.

From now on we leave the field of the sudden spontaneous variants (not connected with visible external stimuli), to reach the field of the gradual adaptive variants, depending on medium conditions. Comparing the latter ones with my idea (widely accepted) on the *constitutive nature of all the enzymes and their division into the habitual* (*customary*) *and adaptive ones* (1940), following the fact that they appear either independently or in dependance of the chemical composition of substrates, or of other conditions, I reached the conclusion that the ambiental factors can carry out biochemical changes neither *accustoming* nor *forcing* the germs to change their enzymatic systems (which fact might lead to an infinite number and quality of deviations from normalcy), but on the contrary *exciting latent enzymes* into the single divergent cells, so that it is quite clear why the variation can be peformed more or less easily and swiftely, according to the number of such cells and their degree of predisposition.

And, therefore, *the adaptative variants and dissociations too stand within the pattern of the normal physiologic phenomena, and they are not exceptional biologic phenomena.*

They also can assume an *apparent* character of stability and inheritance, within the species, in quite the same manner as the true mutants, also for what concerns the retromutations to possible fluctuative or alternant variants (which the same De Vries admits, at least partially). But since, as well as the spontaneous variants, they do not affect all the individuals in the culture being limited only to some of them, so that the cultures are always built up by a majority of *exact* or *perfect* germs, and a minority of *inexact* or *imperfect* ones, the result is that the development of the variants and reversions depends on the setting of numerous isolations, which must be repeated after a long time, *sometimes after years.*

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[1] Sacchetti, Ciferri e Brunetto (Atti Ist. Bot. Briosi, 4, 1934, Pavia) — Aichelburg U. (Giorn. Batter. 13, n. 3, 1934. Boll. Ist. Sierot. Milanese 13, 120, 1934) — Bardsley A. D. (Jour. ot Hyg. 34, 38, 1934) — Callerio C. (Giorn. Batter. 2, 433, 1927) — Edwards S. J. (J. Comp. Path. Ther. 45, 43, 1932) — Murahami K. (Dobl. Bacter. Orig. 101, 377, 1926) — Pagnini U. (Il nuovo Ercolani, 217, 1934) — Palesa e Colarini (Ann. Igiene, 38, 629, 1928) — Ercoli e Molina (Boll. Ist. Sierot. Milanese 1948). ecc.

This is the conclusion I obtained after the many years I spent experimenting on the cultural characters of the acidoproteolytic cocci, which appear to be endowed with a peculiarly sharp variability and dissociability, sometimes hindering their classification.

Considering however that, in spite of all the technical artifices, the enzymatic variability of a microorganism is not without limits and that, according to the modern hereditary doctrine, the individual variants are to be contained within the limits of the intimate constitution of cytoplast, without affecting the genotype, I arrived at the concept of the *genotypicity of microbial enzymes* (1943), which idea, while explaining the reappearing. *also after a long time,* of some biochemical properties, puts a limit to their modifications and has important connections with the same bacterial systematics.

It allows us, indeed, to establish, on one hand, enzymatic species well defined, and, on the other hand, to unite together species showing very close affinity, as enzymatic variants of the same species 1). I like better the term ,,variants'' because one is here dealing with unconstant and reversible changes; on the contrary the terms ,,variety'', ,,breed'', ,,subspecies'' stand for an implicit character of stability.

As a conclusion, because of the principle of the genotypic individual differences, each bacterial culture, *even if monocytogenetic,* is a population of cells endowed with their genotypic enzymatic properties at different degrees, which can produce variants and return, even *after some years,* to the original type, since the variants contain always, altough sometimes in the smallest numbers, cells of different types.

IV — *Specificity of the enzymes.*

This is a question under discussion by two opposite theories. According to Balyss the enzyme is a pick-lock opening several locks; according to Fischer each enzyme is a key fit for only a particular lock, since the enzymes are endowed with the same composition of the substances upon which they act; and the specificity of enzymes does not only affect the three main types of organic substances (glycides, protides, lypides), but it is also bound to the single substances, and sometimes even to a peculiar stereochemical structure of the same substance.

It has been moreover demonstrated that the same enzymatic type is endowed with particular characters according to its derivation, either animal, vegetal or microbial.

Also about these subjects concerning peculiar qualities of the biocatalysators, distinguishing the latter ones from the inorganic catalysts (demonstrating a much larger range of action) the acidoproteolytes gave some contributes, fore runner of the modern enzymology.

About the *specificity of enzymes in relation to the substance,* since as back as in 1902 I demonstrated some acidoproteolytic udder cocci which (contrary to the common belief), though peptonizing the milk do not dissolve gelatine, I gave the first item of the lack of parallelism between collolysis and caseolysis. This observation later has been confirmed and supported by myself and

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[1]) See my special work about the subject in the references.

by others as for the various acidoproteolytes (Wahlin, J. Bact., 1928; Janoscheck, Milchw. Forel, 1931; Corberi, Arch. Mikrob, 1938; Gorbach and others), and it has been enlarged to the alkalinoproteolytes and glycolytes, as the non-dissolving *B. coli*, which, cultured in milk constantly alkaline, appears able to caseolyze (Schierge, Zeit. Exp. Med., 1926).

Among the pathogenic non-dissolving-gelatin but caseolytic germs, I remember here my *B. minimum mammae*(syn. *B.* or *Coryneb. pyogenes bovis*, Reinhardt — Lehrbuch d. tierärztl. Geburtshilfe, 1924), *Pneumococcus* (Gorini, 1923; Avery and Cullen, 1923), *B. typhi* (Gorini, 1926). And we have also items of bacteria dissoving gelatin but not caseolytic (Henneberg).

All the above reported observations have been supported recently by the discovery and the isolation of enzymes separately attacking casein (*caseinases*) or gelatin (*gelatinases*) (Gorbach, Maschmann, Hoogerheide). Besides I detected acidoproteolytes which do not dissolve the common alkaline gelatin but which attack sugared gelatin (either glucosed or lactosed), so that, while in the cultures of the plain proteolytes the sugars (as we already have seen) shelter the protides, in the cultures of acidoproteolytes they might favour the proteolysis; this phenomenon is also made clear to-day by the fact that acidoproteolytes elaborate peculiar proteases, with optimum pH at strong acid reaction (*acidoproteases*); and if some of them attack also the ordinary alkaline gelatin, this is dependent upon their contemporary production of common bacterioproteases, acting at alkaline reaction (Gorbach). The proteasic system of acidoproteolytes, however, is still under examination, since it differs from that of the animal kingdom, as well as from that of the vegetal kingdom, either in regard to pH, or the activators and inhibitors (Gorini, Grassmann, and Schleich, *l. c.*).

It is a matter of fact that the specificity of enzymes comes forth the more, the better are carried out the researches concerning their isolation and the dissociations of the enzymatic systems.

In this connection must be carefully considered the recent works by Maschmann (*l. c.*),according to whom the common dissolving aerobic bacteria elaborate a proteinase which is not fit for the name of gelatinase, but which is a proteasic system of particular type, attacking gelatin, casein and clupein, while on the contrary the enzyme of the anaerobic dissolving bacteria is a true specific gelatinase since it hydrolizes gelatin and glutin, that is only the two artificial products of collagen.

I remember also the researches performed by Flu and Beeuvotles (Zentr. Bact., 1939) about the differences between the collolytic and hemolytic proteases of *Vivrio comma*, and the Imaizumi's works (Journ. Bioch. Japan, 1938) on the proteolytic enzymes of both gelatin-dissolving and non-dissolving bacteria. Barnett Cohen detected differences between hemolysin and other proteases, and even between the lypases and phosphatases of *B. subtilis.* It seems, on the other hand, that no parallelism exist also between the enzymes attacking the different glycides.

With concern to the *specificity of enzymes related to their derivation*, I demonstrated (back in 1892—94) that the chymase of *B. prodigiosum* and other bacteria differs from the animal chymase at least by two points: its heat-resistance and its action on the crude milk; the animal chymase is destroyed at 65°—70° C. and it does not curdle the boiled milk, whereas the bacterial chymase is still active after the permanence of 15′ at 100° C. also in a wet state, and it curdles also the autoclaved milk. These differences have been confirmed later and enlarged by myself and several other authors (Wahlin) and more recently by Tagmara Talce-Niedra (Enzymologia, 1939—1940), who compared the zoochymase with the phytochymase of various plants (ricinus, ficus carica, budding barley, takadiastase and so on) and with the chymase of 230 bacterial species which, tested with my method of ,,milk on agarculture'', appeared able to curdle the milk either without or almost without acidification (pH = 6.5—7).

She discovered that zoochymase and phytobacteriochymase differ above all in their action upon the heated, boiled or autoclaved milk; the latter one is curdled by the phytobacteriochymase but not by zoochymase; the curdling speed of bacteria increases from the autoclaved to the plainly pasteurized and the fresh milk; also in relation to the curdling temperature, the phytobacteriochymases perform better at 60° C. than at 40° C., whereas the zoochymase curdles, at 60° C., very painstakingly; as for acidity, the bacterial chymase has its optimum at pH between 6 and 7, whereas zoochymase between 5 and 6.

To the same results one is led, while confronting the amylases of pancreas, the spittle, the malt, the hyphymycetes and the schyzomycetes, which demonstrate their correspondent optimal pHs respectively at the following values: 6.9 — 6.7 — 4.4 — 3.8 — 8, and their optimal temperature respectively at 40°—50° C. 40° C.; 56°—60° C., 60° C., 80°—85° C. A bacterium can show also individual differences against the same enzyme. Barnett Cohen observed differences among the phosphateses elaborated by the various strains of *B. subtilis.*

Here I remember, because of the analogy, that according to Regnier and al. (C. R. Soc. Biol., 1935) the source of proteins deserves a greater importance than the quality of the enzymes employed by the disgetive processes, for instance pepsin or pancreatin. I desire to remember also the taxonomic (or pertaining to the species) specificity of the proteins used for scientific purposes in the medical and botanical fields to study the affinities among the different species, varities and breeds (Mazzaracchio, Rend. Ist. San. Pubbl., 1938).

V — *The liberation of enzymes.*

For scholastic reasons enzymes get distinguished into *ectoenzymes* (also called *esoenzymes*) and *endoenzymes*, according to their type of liberation, that is, whether they are directly discharged by the living microorganisms into the nutritive medium, or act within the cellular limits. Many authors, however, state that still only the dead or anyway damaged cells could put forth their enzymes (Oppenheimer, 1926; Euler, 1927; Gorbach, Archiv Mikrob, 1930; Moycho, C. R. Ac. Sc., 1935). In order to solve the problem, the research of enzymes in the sterile filtrate of cultures is certainly not sufficient, even if they are very young, since the studies about the bacterial autolysis resulted in the knowledge of its very precocious beginning, so that it is practically impossible to obtain cultures sufficiently developed and in the same time surely free of autolysis.

Anyway to-day the question has got another direction of clearing. Comparative observations performed on young and grown cultures, and on others, either filtered or autolyzed or killed by various and suitable physical and chemical treatments, on the basis of the classic researches made by Willstatter and Rohdewald, led the students to the conclusion that the enzymes are contained within the cell partially in a soluble state, partially in an insoluble state; the first ones, called ,,lyoenzymes'' are easily liberated by the living cells; the second ones, called ,,desmoenzymes'' are liberated only after the death of the cells because of processes of autolysis, hydrolysis or of a different, also mechanical nature, which can be limited to the destruction of the cellular membrane, if the desmoenzymes are simply lying in the protoplast, that is purely *adsorbed*; or else the said processes must deeply destroy the enzymatic *simplex* (symplegma) if the desmoenzymes are chemically bound to the protoplast. This distinction deserves attention both theorical and practical; as a matter of fact the easily dischargeable enzymes are responsible for catalytic reactions negligeable from an energetical viewpoint, and they are of easy preparation; whereas the enzymes which are difficult to discharge are entangled with sharp energetic exchanges, and their extraction needs far more complex methods, either for the scientific or for the practical purposes. For instance, 1 gm. of substrate, under the action of the hydrolasic *lactase* liberates 23 calories, whereas under the action of the clastasic *lactoacidase* it liberates some 82 calories. We must moreover keep in mind the fact that the lyoenzymes can be such because of their true nature, but they can also become such because of an enzymatic transformation, starting from the desmoenzymes. This consideration results in the necessity that a distinction between the enzymatic or autolytic liberation and the one obtained otherwise, drops as useless; it also drops the rigid attribution, hitherto followed, of ectoenzymes to the hydrolases or schyzazes, and of endoenzymes to the desmolases or clastases. The literature reports some items of lyo- and desmoesterases; lyo- and desmoamylases; lyo- and desmoproteases (BAMANN and SALZER, Erg. Enzym., 1938).

It was just in the field of proteases that I first suggested the hypopthesis of the existance of ectoenzymes discharged by the living cell, when back in 1892 I demonstrated that: „*The chymase (proteinase) of B. prodigiosum must not be a product of the cellular autolysis; as a matter of fact the curdling power of the cultures, obtained cell-free by means of filtration, does not increase parallelly to the age of the cultures themselves*". Many years thereafter the problem has been spotlighted by various authors (NICOLLE, Ann. Inst. Pasteur, 1907; OTZUCA, Acta Scholae med. Kyoto, 1916; FLEMMING, Proc. R. Soc., 1922; NEIL, Journ. exp. Med., 1927) and especially by VIRTANEN and TARNANEN (Nat. Wiss., 1931, Zeits. physiol. Chemie 1932); the latter authors, after having kindly remembered my previous researches, studied thoroughly the behaviour of the alkalinoproteolyte *B. fluorescens liquefaciens*, and reached the conclusion that the proteinases are ectoenzymes, whereas the peptidases are endoenzymes; so that they could explain why the mechanism of the bacterial assimilation of protides is carried out gradually in two successive phases; at first the proteins are solubilized abroad, under the action of ectoproteases; the resulting peptides can afterwards enter the cells to be transformed into aminoacids under the action of the endopeptidases.

The same conclusion resulted from the researches performed on the lactic acidoproteolytic cocci (GORINI, GRASSMANN and SCHLEICH, l.c.); these bacteria, moreover, because of their peculiar biochemism, gave another convincing demonstration of the real existence of a difference between the ecto- and endoenzymes, better between the lyo- and desmoproteases; since into the bacterial bodies of *cultures, even if old ones, of Uddercoccus* can be detected the peptidases, and *these only*, whereas in the correspondent cultural liquid, which reached a pH of 4.5, can be practically no more detected neither proteolytic nor proteinasic nor peptidasic activities (LUIGI GORINI, Enzymologia, 1942).

In this way it can be easily explained why also the common plain lactic acid bacteria can produce proteases; but not by the way followed by the acidoproteolytes, since these produce lyo- and desmoproteases, acting during their lifetime, and respectively after their death, on proteins and peptides, whereas the former ones elaborate only desmoproteases, acting after their death only and which do not attack the proteins, but only peptides.

An important difference between the lyo- and the desmoenzymes lays also in the fact that the desmoenzymatic concentration in the cultures appears in direct ratio with the number of cells; on the contrary the lyoenzymatic concentration increases again after the stopping of the cellular multiplications. From the latter consideration it results that the carbohydrasic fermentations depending on desmoenzymes stop when the number of cells has reached its maximum; whereas in the proteasic fermentations depending on lyoenzymatic proteinases appear no relations between the number of cells and the amount of the digested protein (CONSOLE and RAHN, Journ. Bact., 1938). We have shown the practical inference of these observations in the evaluation of the participation of my lactic acidoproteolytic uddercocci in the process of cheese ripening which I have explained through the enzymological bacteriology (Enzymologia, 12, 82, 1947).

Conclusion

The enzymatic tripartition of bacteria into: ,,glycolytes, proteolytes and acidoproteolytes'', which I suggested as back as more than half a century ago on the basis of their metabolism in milk, has been enlarged in 1937 to medical field on the basis of their metabolism in blood serum, substrate equivalent to the milk.

The work on this subject led me to forerunner results, which find sound explanation and support in the recent enzymologic acquisitions, newly enlightening various microbiologic questions, among which above all the nature of microbial enzymes, their different behaviour against the external factors, the different fermentation of the proteic substance, the spontaneous and adaptative biochemical variants and dissociations due to normal individual divergences, the specificity and discharge of enzymes.

This work allowed me to reach the idea of the constitutivity and genotypicity of microbial enzymes, distinguished as habitual (customary) and adaptative ones, enlarging to the bacteria the up-to-date hereditary doctrine, which lends eminent contributions to microbial systematization.

References

Concerning my italian works, see the *Rendiconti Istituto Lombardo di Scienze e Lettere* from 1901 on, where appear cited also the works I published before or elsewhere. Many researches have been reviewed in several international papers. For the ones about the *variations depending on individual divergences*, see: Zentralbl. Bakt., II, 1922; Journal Dairy Science, 1922; Le Lait, 1922; — For the works concerning the *genotypicity of microbial enzymes*, see: Zentralbl. Bakt. II, 1944; Abderhalden's Fermentforschung, 1945; Le Lait, 1944.

Inhibition of pathogenic fungi in vitro by p-Hydroxy Methyl Benzoate

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(6.7.1949)

Introduction

Since the discovery of the inhibitory action of esters of *p*-hydroxybenzoic acid upon fungi by Sabalitschka (Neidig and Burrell, 1944), these compounds have been widely used in the prevention of mold growth. The methyl ester, under a variety of trade names, is commonly employed by biologists in media for rearing the fruit fly, *Drosophila*, for genetical experiments. In such cultures, mold contamination was formerly a troublesome problem. In the presence of 0.1 % p-hydroxy methyl benzoate, mold growth is suppressed.

Neidig and Burrell (1944), in an exhaustive resumé of literature concerning esters of *p*-hydroxybenzoic acid, list 377 references, almost all of which concern studies made in Europe since 1930. The esters of *p*-hydroxybenzoic acid are much more effective against microorganisms than is the free acid. The larger the esterifying group, the greater is the inhibitory power. The esters are only sparingly soluble in water, however, the solubility of the methyl ester being 0.25 % and that of higher homologues even less (Neidig and Burrell, 1944).

Toxicity of these compounds to experimental animals upon oral administration has been studied by a number of workers, notably Schübel and Manger (1929), who found the lethal dose to be extremely large, of the order of 3—6 gm. per kilo.

The combination of high antifungal activity with low toxicity suggested possibilities of successful application in the field of medical mycology. Bonnevie (1936) performed *"in vitro"* experiments with *Epidermophyton*, using the ethyl ester. Favorable clinical results with *p*-hydroxy methyl benzoate (as a 5 % alcoholic solution, or 5 % in an ointment base) were reported by Bang (1937) in the treatment of superficial *Trichophyton* infections. Holm (1944) was likewise successful in the treatment of dermatophytosis of the feet with the ethyl ester, applied in an ointment containing 5 % of the active ingredient.

The present experiments were designed to provide information concerning the amounts of *p*-hydroxy methyl benzoate required to inhibit growth of a number of pathogenic fungi. The methyl ester was chosen because of its greater solubility in water, and consequent potential promise for systemic use.

Materials and Methods

The cultures of fungi were obtained through the courtesy of Dr. Norman F. Conant, Dept. of Bacteriology, Duke University School of Medicine, Durham, N.C. Stock cultures used as sources of inoculum were maintained at room temperature on slants of Sabouraud's agar.

The experiments were performed using Sabouraud's agar medium, supplemented with varying quantities of *p*-hydroxy methyl benzoate, added prior to autoclaving. Plates were poured, inoculated, and examined day by day, the results being recorded after a growth period of 2 weeks at room temperature. Controls consisted of plates of Sabouraud's agar without added *p*-hydroxy methyl benzoate.

Because of the large inherent differences in growth rate among the various fungi studied, the amount of growth on control plates was assigned an arbitrary value of ++++. Determinations of growth in the presence of *p*-hydroxy methyl benzoate were recorded, after 2 weeks, on a scale of 0, +, ++, +++, ++++, in comparison with the appropriate controls. In experiments with *Coccidioides immitis*, test tubes were substituted for the Petri plates used with all other organisms.

Results

Several replications of experiments using *p*-hydroxy methyl benzoate in concentrations of 0.0125 %, 0.025 %, 0.05 %, and 0.1 % gave the results shown in Table I.

TABLE I. *Groth of Pathogenic Fungi in the Presence of Various Concentrations of p-hydroxy methyl benzoate.*

	p-Hydroxy Methyl Benzoate			
	0.0125 %	0.025 %	0.05 %	0.1 %
Epidermophyton floccosum . .	++	0	0	0
Microsporum audouini	++	0	0	0
Microsporum canis	++++	0	0	0
Microsporum gypseum	++	0	0	0
Trichophyton ferrugineum . .	++++	0	0	0
Trichophyton mentagrophytes .	++++	++++	0	0
Trichophyton rubrum	++	0	0	0
Trichophyton tonsurans. . . .	++	+	0	0
Hormodendrum compactum . .	++++	0	0	0
Hormodendrum Pedrosoi . . .	++++	0	0	0
Phialophora verrucosa	++++	0	0	0
Geotrichum sp.	++++	++++	0	0
Monosporium apiospermum. .	++	+	+	0
Nocardia asteroides	++++	0	0	0
Sporotrichum schenckii. . . .	++++	+	0	0
Blastomyces dermatitidis . . .	++++	+	0	0
Candida albicans	++++	++++	++++	0
Cryptococcus neoformans . . .	++++	+	0	0
Coccidioides immitis	++	0	0	0
Haplosporangium parvum . .	++	0	0	0
Histoplasma capsulatum . . .	+	0	0	0

Discussion

Perhaps the most significant result of these experiments is the finding that all of the organisms tested, including those associated with subcutaneous and systemic mycoses, as well as the dermatophytes, are inhibited by the drug in concentrations of 0.1 % or below. The methyl ester of *p*-hydroxy benzoic acid would thus appear to a compound with general antimycotic activity. When the inhibitory concentrations of *p*-hydroxy methyl benzoate are considered in relation to limits imposed by the solubility of the compound and by its toxicity to animals, it seems apparent that this material is worthy of further study in relation to its possible use in the treatment of mycotic diseases.

Summary

p-hydroxy methyl benzoate is fungistatic, in rather low concentrations, to pathogenic fungi. 0.1 % *p*-hydroxy methyl benzoate was required to inhibit growth of *Candida albicans* and *Monosporium apiospermum* on a Sabouraud's agar medium. *Trichophyton mentagrophytes, T. tonsurans, Geotrichum* sp., *Sporotrichum schenckii, Blastomyces dermatitidis* and *Cryptococcus neoformans* failed to grow in the presence of 0.05 % *p*-hydroxy methyl benzoate. Growth of *Epidermophyton floccosum, Microsporum audouini, M. canis, M. gypseum, Trichophyton ferrugineum, T. rubrum, Hormodendrum compactum, H. Pedrosoi, Phialophora verrucosa, Nocardia asteroides, Coccidioides immitis, Haplosporangium parvum* and *Histoplasma capsulatum* was suppressed by 0.025 % but not by 0.0125 % of this compound.

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Probabili sinonimi di Allescheria Boydi

(= Monosporium apiospermum)

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La recente constatazione di Emmons (Mycologia, **36** (2), 188—193. 1944) che il *Monosporium apiospermum* Sacc. (1911) è la forma conidica di *Allescheria Boydi* Shear (1922), permette riportare alla specie i seguenti sinonimi:

1) = *Scedosporium apiospermum* Sacc. (1913)
2) = *Monosporium sclerotiale* Pepere (1914)
3) = *Monosporium nigricans* Pepere (1914)
4) = *Monosporium apiospermum* var. *sclerotiale* Pepere (1914)
5) = ? *Scedosporium* sp. De Magalhaes (1919)
6) = *Aleurisma apiospermum* Main (1921)
7) = *Cephalosporium Boydii* Shear (1922)
8) = *Dendrostilbella Boydii* Shear (1922)
9) = *Glenospora Boydii* Pollacci et Nannizzi (1928)
10) = *Indiella americana* Delamare et Gatti (1929)
11) = ? *Scedosporium Magalhaesi* Fraés (1930)
12) = ? *Macrosporium Magalhaesi* Dodge (1935)
13) = *Glenospora viridobrunnea* Red. et Cif. (1942)

Data la perfetta concordanza della morfologia della forma conidica delle *Glenospora viridobrunnea* Red. et Cif. (1) (in Redaelli e Ciferri, Granul. Fungine Tropic., p. 534—536, fig. Firenze, 1942) e l'identità del caso clinico con quello classico del Pepere da *Monosporium apiospermum*, riteniamo che la specie (da loro descritta „ad interim'' e salvo comparazione con le specie affini) sia da riportare a *M. apiospermum*, da cui differirebbe quasi solo per la pigmentazione del substrato di cultura in bruno, carattere che, col tempo, si attenua e quindi si perde. E' quindi solo un ceppo appena aberrante da quello tipico, forse senza valore sistematico, tanto più che non sappiamo se, di recente isolato, pigmenti egualmente i terreni di cultura.

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[1] Diagnosi latina in Redaelli e Ciferri, Atti Ist. Bot. Lab. Critt. Univ. Pavia, V, **3** (1), 25 (1943).

The fungistatic activities of vitamin K on dermatophytes

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(7.8.1949)

Vitamins K belong to the group of quinones, the fungicidal properties of which were the subject of a patent issued in 1926 to BRODERSEN and EXT (1930). TER HORST and FELIX (1943) described the fungicidal properties of 2,3-dichloro-1,4-naphthoquinone on *Pythium, Rhizoctonia, Glomerella, Chaetomium, Metarrhizium, Stachybotrys, Aspergillus, Penicillium.* Tetrachloro-p-benzoquinone was patented as a fungicide by TER HORST (1944). VINET (1945) reported the inhibitory effect of 2-methyl-naphthoquinone, 2-chloro-naphthoquinone, and 2-chloro-3-hydroxy-naphthoquinone on *Aspergillus niger.* WOOLEY (1945) found that vitamin K prevents the antifungal action of 2,3-dichloro-1,4-naphthoquinone on *Saccharomyces cerevisiae* and *Endomyces vernalis.* GONZÁLEZ (1945) described the inhibitory effects of 2-methyl-1,4-naphthoquinone, hydroquinone, and benzoquinone on *Penicillium notatum.* COLWELL and MCCALL (1946) reported the fungicidal and fungistatic concentrations of 2-methyl-1,4-naphthoquinone and 2-methyl-3-methoxy-1,4-naphthoquinone on *Aspergillus niger* and mold F.P.L. no. 517. DONATELLI and DAVOLI (1946) have shown that 2-methyl-1,4-naphthohydroquinone retards the growth of *Penicillium notatum.* 2,3-dichloro-1,4-naphthoquinone or U.S.R. no. 604 (CROSIER, 1946; DAINES and HOPPERSTEAD, 1946) and tetrachloro-para-benzoquinone or Spergon (CROSIER, 1946) have been used as fungicides in agriculture.

Until then the inhibitory effect of quinones on dermatophytes had not been investigated. The first studies of this subject seem to have been those of COLLINS, MCGAVACK and BOYD (1946); they reported the action of tetrachloro-para-benzoquinone *in vitro* and *in vivo* on dermatophytes. OSTER and GOLDEN (1948) investigated the minimum fungicidal concentrations of 11 derivatives of benzoquinone, 20 derivatives of naphthoquinone, and 24 quinoline derivatives on *Trichophyton mentagrophytes.* PRATT, SAH, DUFRENOY, and PICKERING (1948) found the minimum concentration of vitamin K5 which prevents growth of *Trichophyton mentagrophytes, Microsporum canis, M. Audouini, Saccharomyces cerevisiae, Penicillium notatum,* and *Botrytis allii.* MCGAVACK, WEINER, LO CASCIO, BELL, and BOYD (1949) described recent cases of dermatomycoses treated with tetrachloro-para-benzoquinone. OSTER and GOLDEN (1949) reported the clinical use of 8-hydroxyquinoline.

The mechanism of the antifungal action of quinones is not yet definitively explained. COLWELL and MCCALL (1945) investigated the antibacterial action and later on stated that "the mechanism of antibacterial and antifungal action of 2-methyl-1,4-naphthoquinone is by blocking -SH enzymes or metabolites essential for growth. The inhibitory potency of methoxyquinone, however, even in the presence of sulfhydryl compounds, and its failure to interfere with -SH tests indicate either, that the methoxyquinone, in spite of its structural similarity, functions by a different mechanism, or that the foregoing explanation for the activity of 2-methyl-1,4-naphthoquinone is incomplete." (Colwell and McCall, 1946). These conclusions were confirmed by the experiences of Page and Waller (1946). The activity of vitamins K would depend upon the high oxidation-reduction potential of certain quinones (Schönberg, Moubasher, and Mostafa, 1948; Pratt, Sah, Dufrenoy, and Pickering, 1948) and the inhibition of respiratory processes (Ball, Anfinsen, and Cooper, 1947). Hoffman-Ostenhof (1947) suggests that the antibiotic action of quinones is due to a complex mechanism.

Experimental studies

In our experiments attempting to determine the fungistatic activities of vitamin K, the following strains of dermatophytes were used: *Microsporum canis* no. 2.537; *Epidermophyton floccosum* no. 2.069; *Trichophyton mentagrophytes* no. 2.552, and *Trichophyton Schönleinii* no. 1.432. They were isolated from human lesions and belong to the Collection of the Oswaldo Cruz Institute.

Four commercial preparations of vitamin K were compared: *I* (2-methyl-1,4-naphthohydroquinone tetrasodium salt of diphosphoric ester 10 mg., sodium bisulfite 5 mg., and distilled water q.s.p. 1 ml.); *II* (2-methyl-1,4-naphthohydroquinone tetrasodium salt of diphosphoric ester 10 mg., sodium metabisulfite 1 mg., sodium chloride 4 mg., and distilled water q.s.p. 1 ml.); *III* (4-amino-2-methyl-1-naphthol chloride 1 mg., solvent 1 ml.); *IV* (2-methyl-1,4-naphthoquinone 1 mg., 30 % glucose solution 5 ml.).

Sabouraud's medium, both liquid and solid, with glucose were utilized. The cultures remained at room temperature, and were examined every day from the 4th. to the 30th. day after seeding.

Results.

Table 1 lists the results.

It may be observed that the fungistatic activities of the vitamins K are higher in the liquid than in the solid medium.

Table 1. *Maximum nonfungistatic (+) and minimum fungistatic (—) concentrations of vitamins K, in mg %, on dermatophytes, using Sabouraud's medium, both liquid and solid, with glucose.*

		Microsporum canis		*Epidermophyton floccosum*		*Trichophyton mentagrophytes*		*Trichophyton Schönleinii*	
		+	—	+	—	+	—	+	—
Solid medium	I	16,6	25,0	10,0	16,6	10,0	16,6	10,0	16,6
	II	9,1	20,0	4,8	9,1	20,0	33,0	20,0	33,0
	III	4,8	9,1	4,8	9,1	20,0	33,0	20,0	33,0
	IV	4,0	6,6	1,8	4,0	1,8	4,0	1,8	4,0
Liquid medium	I	50,0	100,0	—	0,2	1,5	3,1	3,1	6,2
	II	50,0	100,0	12,0	25,0	50,0	100,0	50,0	100,0
	III	0,6	1,2	0,6	1,2	2,5	5,0	0,6	1,2
	IV	0,5	1,0	0,25	0,5	0,5	1,0	0,25	0,5

Vitamin *IV* exhibited the highest fungistatic activity on all the dermatophytes in both, the solid and the liquid medium. The fungistatic activities of the other vitamins K vary, in each medium, according to the dermatophyte.

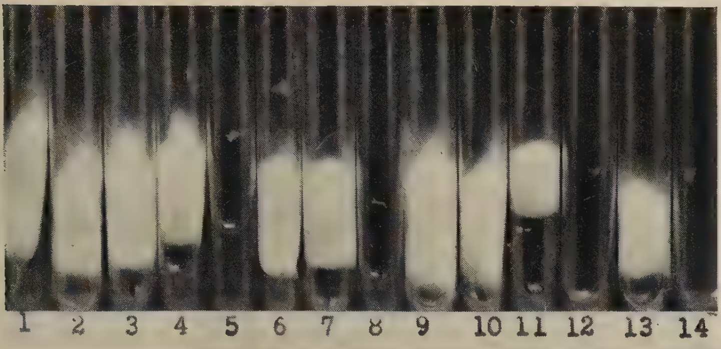

Fig. 1 — Action of vitamins K on *Microsporum canis* no. 2.537, 14 days after seeding. (1—4) Vitamin I from 2,38 to 16,6 mg %, (5) idem 25,0 mg %; (6—7) Vitamin II from 4,76 to 9,1 mg %, (8) idem 20,0 mg %; (9—11) Vitamin IV from 0,95 to 4,0 mg %, (12) idem 6,6 mg %; (13) Vitamin III 4,8 mg %, (14) idem 9,1 mg %.

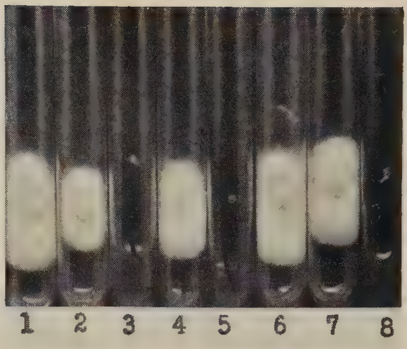

Fig. 2 — Action of vitamins K on *Epidermophyton floccosum* no. 2.069, 14 days after seeding. (1—2) Vitamin I from 2,38 to 10,0 mg %, (3) idem 16,6 mg %; (4) Vitamin II 4,8 mg %, (5) idem 9,1 mg %; (6—7) Vitamin IV from 0,95 to 1,8 mg %, (8) idem 4,0 mg %.

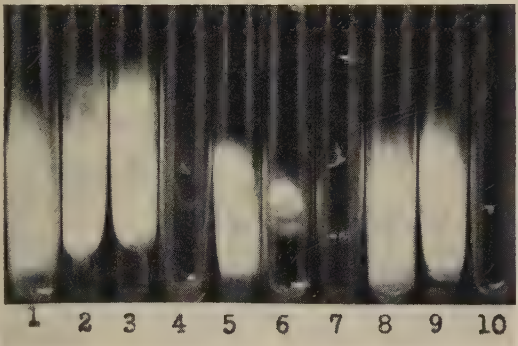

Fig. 3 — Action of vitamins K on *Trichophyton mentagrophytes* no. 2.552, 14 days after seeding. (1—3) Vitamin I from 2,38 to 10,0 mg %, (4) idem 16,6 mg %; (5—6) Vitamin II from 9,1 to 20,0 mg %, (7) idem 33 mg %; (8—9) Vitamin IV from 0,95 to 1,8 mg %, (10) idem 4,0 mg %.

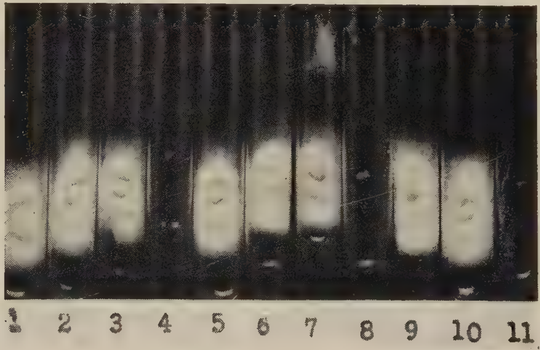

Fig. 4 — Action of vitamins K on *Trichophyton Schönleinii* no. 1.432, 14 days after seeding. (1—3) Vitamin I from 2,38 to 10,0 mg %, (4) idem 16,6 mg %; (5—7) Vitamin II from 4,76 to 20,0 mg %, (8) idem 33,0 mg %; (9—10) Vitamin IV from 0,95 to 1,8 mg %, (11) idem 4,0 mg %.

In solid medium: on *M. canis* (Fig. 1) after vitamin *IV* the most active are, successively, vitamins *III, II*, and *I*; on *E. floccosum* (Fig. 2), *T. mentagrophytes* (Fig. 3), and *T. Schönleinii* (Fig. 4) vitamins *III* and *II* were equally active and more so than vitamin *I* when used on *E. floccosum*. In liquid medium, vitamin *III* is more active than *I* and *II* on *T. Schönleinii* and *M. canis*, but on *E. floccosum* and *T. mentagrophytes* the most active are, successively, vitamins *I, III*, and *II*. The low fungistatic activities of vitamins *I* and *II* may be caused by the presence of sodium bisulfite (Hoffman-Ostenhof, 1947).

In solid and liquid medium, *E. floccosum* was the most sensitive to all the vitamins K. In solid medium, *T. mentagrophytes* and *T. Schönleinii* exhibited the same sensitivity to all the vitamins K.

Summary and conclusions

Four commercial preparations of vitamin K were evaluated for their fungistatic activities on the following dermatophytes: *Microsporum canis, Epidermophyton floccosum, Trichophyton mentagrophytes*, and *Trichophyton Schönleinii*.

Table 1 lists the maximum nonfungistatic and the minimum fungistatic concentrations of each vitamin K.

The fungistatic activities of the vitamins K are higher in the liquid than in the solid medium.

Vitamin *IV* was the most active on all the dermatophytes in both, the solid and the liquid medium. The fungistatic activities of the other vitamins K vary, in each medium, according to the dermatophyte. Sodium bisulfite decreases the fungistatic activities of vitamin K.

Epidermophyton floccosum was the most sensitive to all the vitamins K used.

Acknowledgment

The authors wish to express their thanks to Laboratórios Eli Lilly & Co., Productos Roche Químicos e Farmacêuticos S.A., Parke Davis & Co., and Laborterápica S.A. for supplying the vitamins K used in this study.

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Biotin - an essential growth factor for the filamentous pathogenic fungus *Allescheria Boydii* Shear[1]

by

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(27.12.1948)

In the course of a study on the growth factor requirements of pathogenic fungi we were able to observe that *Allescheria Boydii* (no 1699) requires biotin for its development. This pathogenic fungus was isolated by BOYD and CRUTCHFIELD from a mycetoma of white grains (2) and studied as well as described by SHEAR (6).

According to NICKERSON and WILLIAMS in a recent review on the growth factor requirements of pathogenic fungi "Biotin has not been shown to be a growth factor for any of the filamentous pathogenic fungi . . ." (3). SCHOPFER and BLUMER (4, 5) reported that a strain of *Trichophyton album* required biotin in determined conditions, depending on the age of the culture and composition of the medium (nitrogen source). They therefore concluded that biotin is a complementary growth factor for the strain of *T. album* studied (5).

In our experiments we have used a basal medium similar to that of SCHOPFER and BLUMER and containing per liter: dextrose 30 g, asparagine (recrystallized) 2 g, KH_2PO_4 1.5 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, agar (purified) 12 g, and trace elements (B, Cu, Fe, Mn, Zn, Mo). More details of the composition of this medium will be reported in a future publication (1).

In order to know the effect of several vitamins (thiamine, riboflavin, pyridoxin, nicotinic acid, calcium panthotenate, inositol, and biotin) on the growth of *A. Boydii*, ten different kinds of media were prepared and selected as follows: basal medium without vitamins, basal medium plus all vitamins, basal medium plus all vitamins and 1 % yeast extract, and the seven other media containing all the vitamins except one (see Fig. 1). The fungus was planted and the tubes were maintained at room temperature. No growth was observed in those tubes in which biotin was not added, namely tubes 1 and 8 (Fig. 1).

When ammonium sulfate or ammonium citrate were used as a nitrogen source instead of asparagine, the same results were observed, i. e., no growth occurred in the media without biotin. With ammonium sulfate, even in the presence of biotin, the growth was poorer than that observed in the cultures supplied with ammonium citrate or asparagine, thus indicating that the former nitrogen source is less assimilable by the fungus.

To know whether the growth of *A. Boydii* would be proportional to the

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[1] The strain of A. BOYDII used in the present work was kindly supplied by Dr. P. NEGRONI from the Instituto Bacteriologico de Buenos Aires, and according to the information given by Dr. NEGRONI it was originally received from the Laboratory of Dr. CHARLES THOM. U. S. D. A.

The author wishes to express his appreciation to Drs. A. E. DE ARÊA LEÃO and GILBERTO G. VILLELA for their interest in connection with this work.

concentration of the biotin present in the medium, experiments were performed by using the same basal medium without agar. The medium was distributed in amounts of 10 ml in Erlenmeyer flasks of 50 ml capacity. Biotin was added in graded amounts from 0.0001 to 0.1 μg per flask. The flasks were seeded with a suspension of spores and maintained at room temperature during 11 days. They were then sterilized and the cultures filtered through a sintered glass filter, washed with distilled water, dried during 4 hours at 100° C, and then weighed. Table 1 shows the results obtained. A close proportionality of the weight and the biotin present is shown with the amounts of 0.0001 to 0.01 μg. Up to this amount the growth is steady.

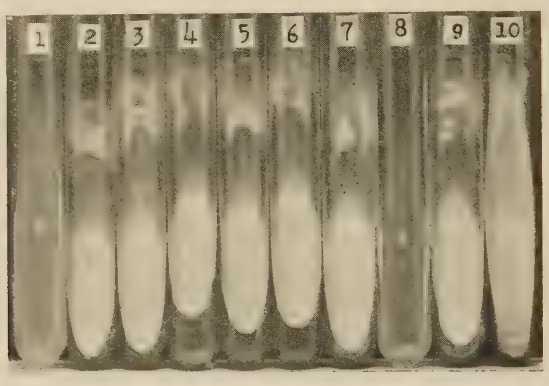

Fig. 1. *Allescheria Boydii* (1699) grown on basal mineral-dextrose medium containing asparagine and purified agar, with varied supplements of vitamins and yeast extract (after 50 days). (1) basal medium without vitamins; (2) minus thiamine; (3) minus riboflavin; (4) minus pyridoxin; (5) minus nicotinic acid; (6) minus calcium pantothenate; (7) minus inositol; (8) minus biotin; (9) plus all vitamins; (10) plus all vitamins and 1 % yeast extract.

We may conclude that in the conditions of our experiments, biotin is an essential growth factor for the strain of *A. Boydii* studied. More detailed studies on the nutrition of this organism as well as the possibility of its use for the assay of biotin are in course.

TABLE I

Effect of the amount of biotin on the culture of *Allescheria Boydii* (1699) after 11 days growth in 10 ml of basal mineral-dextrose medium containing asparagine.

	Biotin 10^{-3} μg.									
	0	0.1	0.2	0.5	1	5	10	20	50	100
Dry weight of mycelium mg.	0	9	11	21	30	48	63	67	65	61

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NOTE: This paper was in press when SALVIN (J. Inf. Dis., 1949, **84**, 275—283), reported that biotin was necessary for the development of the yeastlikephase of *Histoplasma capsulatum,* and that only one strain of six strains tested required biotin for the mycelial growth.

Nueva observacion de histoplasmosis en la Republica Argentina

Por el

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(Laboratorio del Instituto de Clínica Quirúrgica. Buenos Aires)

(13.1.1949)

La observación que paso a estudiar representa la 7a. de ésta enfermedad parasitaria estudiada en nuestro país en un lapso de 10 años, con la particularidad de que todos éstos casos presentáron — aparentemente — lesiones localizadas en el revestimiento cutáneo, mucoso o cutáneo-mucoso, sin participación del sistema retículo-histiocitario visceral.

A la bibliografía argentina citada al estudiar un caso anterior en colaboración con Di Pietro (1), debemos agregar la de los Dres. Ferradás, Bonfiglioli y Fayt (2), publicada el año pasado en los Archivos Argentinos de Tisiología, en un español con 8 años de residencia en el país, habiendo vivido anteriormente en el Paraguay y, dentro del territorio de la República, en las provincias de Corrientes y de Tucumán.

Historia Clínica:

Enrique R.A., argentino, casado de 59 años de edad, comerciante; domiciliado en la Capital Federal. Concurre al C.A.C. del Instituto de Clínica Quirúrgica con el presunto diagnóstico de neoplasia, donde queda inscripto con el N° de R.G. 122.887 y al cuidado del Dr. Enrique Viacava (Abril 14/947).

Enfermedad actual — Empieza hace 4 meses por una especie de afta situada en la cara interna de la comisura labial del lado izquierdo, que se rompe dejando salir un líquido como agua; posteriormente queda una ulceración no dolorosa pero molesta, aumentando progresivamente de tamaño y en profundidad. Desde hace 8 meses y en forma periódica, se infartan algunos ganglios del cuello, también del lado izquierdo. Estos ganglios aumentan y disminuyen de tamaño.

Hace 4 años se sintió decaído, con pérdida de peso y de fuerzas. Un examen radiológico practicado en esa época mostró en ambos campos pulmonares, una diseminación de sombras nodulares pequeñas que hiciéron suponer al médico tratante en la existencia de una tuberculosis, razón por la cual lo envía a Córdoba, permaneciendo en la vecindad de Río IV°. Repetidas baciloscopías, fuéron siempre negativas para el bacilo de Koch.

Después de 4 años de permanéncia en Córdoba, regresa a la Capital Federal y neuvas radiografías no muestran ninguna modificación de su proceso pulmonar.

Antecedentes hereditarios — Sin importancia.

Antecedentes personales — Gastrectomizado por úlcera duodenal.

Estado actual (Abril 14/947) — Sujeto normolíneo, en regular estado de nutrición. Presenta en la cavidad bucal las siguientes alteraciones:

En la cara mucosa de la comisura labial del lado izquierdo, se observa una profunda ulceración en ángulo diedro, con fondo granulante y de casi 1 ctm. de diámetro, poco infiltrante, poco dolorosa, cubierta por exudado de color blanco-amarillento. En la comisura misma, se observa una fisura que sangra con facilidad, y, en la semi-mucosa labial, en la vecindad de ésta lesión, una descamación seca, blanca y adherente, (fig. 1). Uvula, con base edematosa y roja. En amigdala del lado izquierdo, se observa un nódulo blanco-amarillento, como una arveja: Amigdalítis criptica?

En el lado izquierdo del cuello, se palpa una adenopatía irregular, dura, dolorosa a la presión, poco movible; parece estar vinculada al esternocleido-mastoideo.

Aparato respiratorio: Disminución del murmullo vesicular y cierto grado de dispnea.

Abdomen: Presenta la cicatríz de su antígua gastrectomía. Nada de particular a la exploración semiológica.

Resto del organismo: Nada de particular.

Con el diagnóstico clínico de epitelioma, se le practica en el C.A.C. una biopsia de la ulceración cuyo examen histopatológico (Inclusión N° 19904) muestra tratarse de una Histoplasmosis; diagnóstico confirmado por otra biopsia (Inclusión N° 19924) y por examen de frotis por impresión previamente coloreado con May Grünwald-Giemsa, que pusiéron en evidencia formas del *Histoplasma capsulatum.*

Exámenes complementarios:

El enfermo posée numerosas radiografías de tórax cuyas cópias fotográficas correspondientes a placas tomadas en Oct. 22 de 1943, Mayo 19 de 1947 y Octubre 3 de 1947, se reproducen por ser las más demostrativas. En todas ellas se observan sombras micronodulares diseminades en ambos campos pulmonares, (figs. 2, 3 y 4).

Exámenes de sangre practicados en laboratorios privados, muestran la existencia de una anemia hipocrómica más o menos apreciable.

Biopsia de lesión de cara interna de mejilla practicada por el Dr. Viacava (Inclusión N° 19904; Abril 15/947) — Hiperplasia papilomatosa. Retículohistiocitosis parasitaria. Se observa gran cantidad de elementos que por su morfología semejan Leishmanias o Histoplasmas. (Dr. Niño).

Raspado de lusión ulcerosa de la boca (Abril 15/947—Dr. Niño) — En el examen microscópico de frotis coloreados se observan escasos *Histoplasma capsulatum,* (fig. 5), libres y en el citoplasma de grandes monocitos. No se observan Leishmanias.

Biopsia de ulceración de mejilla practicada por el Dr. Viacava en su parte profunda (Inclusión N° 19924) — Retículo-histiocitosis parasitaria por *Histoplasma capsulatum.* (Dr. Niño, Abril 18/947).

Inoculación al cobayo y cultivos — Parte de la biopsia practicada anteriormente, se machaca en mortero estéril y se suspende en solución fisiológica para efectuar cultivos sobre Sabouraud glucosado e inoculaciones al cobayo por vía intraperitoneal. Se prolonga la observación de los cultivos y de los animales inoculados por espacio de 45 días, siendo negativos los cultivos como también el examen de los órganos para el *Histoplasma capsulatum* y para el bacilo de Koch. (Dr. Niño, Abril 15/947).

Evolucion:

Se somete al enfermo a tratamiento con Sulfadiazina.

Junio 14/947 — Lesión bucal cicatrizada totalmente. Lesión amigdalina curada. La adenopatía cervical ha desaparecido. Hígado y bazo, aumentado de tamaño y doloroso. Un examen radiológico muestra aumento de las sombras pulmonares en cantidad y en tamaño. No hay expectoración. Persiste la dispnea de esfuerzo. Buen estado general. Hasta la fecha ha tomado 176 grs. de Sulfadiazina.

Se queja de molestias post-prandiales con sensación de angustia y de asfixia especialmente después de la ingestión de alimentos líquidos, (Enfermo gastrectomizado sin régimen adecuado). Dispepsia flatulenta. Se le recomienda una alimentación fraccionada y pocas cantidades de líquidos; practicar un examen parasitológico de matérias fecales e ingerir 1 gr. de Yoduro de potasio diario con el objeto de provocar la expectoración del paciente y la búsuqeda ulterior del bacilo de Koch.

El enfermo se ausenta a Córdoba hasta fines de Diciembre de 1947, sin sumplir las prescripciones anteriores.

Dic. 29/947 — Después de 3 meses sin tomar Sulfadiazina y habiendo sufrido una grippe y un ataque de colitis, me es enviado otra vez por el Dr. Viacava, con manifiesta astenia e inapetencia. Hace dos días el Dr. Rechnieswski le practica un examen hematológico que dá el siguiente resultado:

Hemoglobina (Helliges)	70 %—10,15 grs. %
Glóbulos rojos	3,700,000 × mm³
,, blancos	7,900 × mm³
Valor globular	0,94
Volúmen globular	34
Reticulocitos	30,000 × mm³
Plaquetas	246,000 × mm³

Fórmula leucocitaria:	%	× mm³
Polinucleares basófilos	—	—
,, eosinófilos	1—	79,00
Mielocitos neutrófilos	—	—
Metamielocitos neutrófilos	—	—
Polinucleares neutrófilos (con núcleo en barra)	4—	316,00
,, ,, (con núcleo segment)	56,5	4474,00
Linfocitos	29,5	2320,00
Monocitos	9—	711,00
	100 —	7900,00

Oligocitosis y oligocromenia.-Monocitosis.

De su ant gua lesión bucal no existen rastros. En la pared posterior de la faringe y en la úvula se observa una faringitis granulosa con sensación de sequedad y moléstia. En la base de la léngua se observa una granulación. Ha perdido 3 kilos de peso; se encuentra subfebril y tiene sudores nocturnos.

Se prescribe Metazinol "Wander" 5 grs. diarios; extracto hepático; reticulogen; alimentación fraccionada y 10 gotas diarias de la solución de Yoduro de potasio y sodio al 50 % para provocar la expectoración a fin de realizar un examen baciloscópico.

El enfermo trae una radiografía que meustra una imágen semejante a las anteriores.

Enero 7/948 — Se consigue una escasa expectoración, cuyo examen microscópico no muestra la presencia de bacilos de Koch ni de elementos parasitarios. Escasos micrococcus catarrhalis. Con la expectoración, se inocula por vía intraperitoneal al cobayo N° 40 A, de 300 grs. de peso. — El 29 de Enero de 1948, se sacrifica al animal por inhalación clorofórmica.

Autopsia: Lesión ulcerosa chancriforme en el lugar de la inoculación dando salida a una substancia caseosa. Adenopatías inguinales y lumbares con caseificación. Adenopatías preaórticas y mediastínicas del volúmen de una arveja. Bazo grande y con granulaciones miliares. El examen microscópico de frotis practicados con los ganglios y con la lesión cutánea, muestran una regular cantidad de bacilos de Koch y ausencia de elementos fúnguicos.

A partir de este momento, no tenemos más notícias del enfermo.

Estudio Parasitologico

El estudio parasitológico de la presente observación está basado en el examen microscópico de frotis coloreados con May Grünwald-Giemsa y en el examen histopatológico de las biopsias practicadas sobre la lesión ulcerosa de la mucosa bucal.

Examen histopatológico (Inclusión No 19904 y 19924, correspondientes a biopsias practicadas los días 15 y 18 de Abril de 1947. Coloraciones con hematoxilina eosina y con hematoxilina férrica-eosina).

El examen microscópico de ambos materiales obtenidos respectivamente, del borde y de la profundidad de la lesión, muestra en una visión topográfica una hiperplasia papilomatosa del revestimiento mucoso con rotura y destrucción en algunos sítios. Por debajo se observa un proceso de proliferación histiocitaria denso y con aspecto micronodular, (fig. 6). Examinadas estas estructuras con aumentos más fuertes, muestran estar constituídas histológicamente por 1-2-3 células gigantes multinucleadas con núcleos en corona o conglomerados hacia el centro de la célula; células epitelioídes y otros elementos histiocitarios productos de una intensa proliferación. Fibroblastos y estrechas bandas de tejido conjuntivo tienden a limitar estas formaciones nodulares. La exudación leucocitaria es casi nula y sólo se observan diseminados muy pocos linfocitos y polinucleares neutrófilos. La vascularización es muy discreta.

Los exámenes con aumentos más fuertes, sobre todo de aquellos preparados teñidos con hematoxilina férrica-eosina, permiten observar una cantidad — a veces considerable — de pequeños elementos redondeados u ovoidales, rodeados por una cápsula que no se colorea, que miden término medio, unos 3 μ de diámetro y que por su morfología son semejantes al *Histoplasma capsulatum* Darling, 1906; siendo clasificados como tal. Estos elementos parasitan las células gigantes y otras formaciones histiocitarias como puede verse en las figs. 7-8-9 y 10.

Diagnóstico histopatológico: Retículo-histiocitosis parasitaria por *Histoplasma capsulatum* Darling, 1906. — Dr. Niño.

Examen de frotis por impresion y del raspado de las lesiones — El material previamente coloreado con May Grünwald-Giemsa, muestra en el examen microscópico la presencia de elementos parasitarios en escasa cantidad, algunos libres y otros en el citoplasma de grandes células mononucleadas, representados por corpúsculos de forma esférica u ovoidea, de unos 3 μ de diametro, rodeados por una cápsula incolora bien visible y presentando en su interior una masa cromática que se tiñe intensamente en azul-violado, (fig. 5). En ningún momento pudo observarse nada que parezca un blefaroplasto y que pudiera hacer pensar en la existancia de leishmanias.

Por las razones apuntadas y por la morfología del parásito, éste es clasificado como *Histoplasma capsulatum.*

Comentario

Esta nueva observación argentina de histoplasmosis, diagnosticada por el frotis y el examen histopatológico de biopsias, se prestó como se ha visto a traves de la historia, al diagnóstico clínico de neoplasia, como suele ocurrir en otras micosis de la boca y de sus anexos, error, aún más justificado si se piensa que la lesión primitiva fué seguida de una adenopatia por demás sospechosa, en lo que a manifestaciones locales se refiere, y, de un desmejoramiento del estado general con astenia y pérdida de peso. Pero, si el problema etiológico de sus lesiones locales quedó prontamente aclarado con la intervención del laboratorio, no ocurrió lo mismo con sus lesiones pulmonares que, si bien fuéron sospechadas por otros colegas como de naturaleza tuberculosa, la circunstancia de reiterados exámenes negativos en la búsqueda del bacilo de Koch y el fracaso de la cura de clima, hizo que atribuyéramos las lesiones que se exteriorizaban en las placas radiológicas al *Histoplasma capsulatum,* toda vez que, como se sabe, alteraciones semejantes del parnéquima pulmonar han sido descriptas en la histoplasmosis o enfermedad de Darling. El beneficio obtenido por el enfermo con la sulfamidoterapia (algo más de 176 grs. de sulfadiazina) evidente en lo que se refiere a sus lesiones mucosas y ganglionares y sólo relativo en las modificaciones pulmonares, nos autorizaba a esta interpretación del cuadro clínico presentado por nuestro paciente; sin embargo, la marcada astenia, el desmejoramiento del estado general acompañado de inapetencia y de un estado subfebril con sudores nocturnos, nos llevó a buscar — una vez más en la escasa expectoración provocada, la confirmación de la etiologia tuberculosa tan largamente sospechada. En efecto, la tuberculización del cobayo inoculado con ésta expectoración constituye, sin duda alguna, un argumento de gran valor en la interpretación final del cuadro clínico-radiológico presentado por nuestro enfermo: Por un lado, una granulia pulmonar que sigue su evolución y, por el otro, una histoplasmosis de la mucosa bucal con repercusión ganglionar que cura definitivamente con la medicación establecida.

Antes de terminar, séame permitido recordar que en esta observación, como en algunas otras, no fué posible obtener cultivos del hongo productor de esta parasitosis — el *Histoplasma capsulatum* — posiblemente, porque la abundante y variada flora bacteriana agregada, al invadir rápidamente los médios de cultivo, anulaba e impedía todo desarrollo del hongo en cuestión. Quedan pues, como argumentos para fundar nuestro diagnóstico etiológico de histoplasmosis o retículo-histiocitosis parasitaria, el examen de los frotis y el de las biopsias practicadas que nos muestran a los parásitos con su morfología y afinidades tintoriales características.

Conclusiones

Se efectúa el estudio clínico y parasitológico de una nueva observación de histoplasmosis, retículo-histiocitosis parasitaria o Enfermedad de Darling por *Histoplasma capsulatum* Darling, 1906.

Se trata de un paciente de 59 años de edad con lesiones bucales y ganglionares en el cual coexiste, además, una granulia tuberculosa pulmonar bilateral.

El tratamiento con Sulfadiazina curó sus lesiones histoplasmósicas.

Se trataría de la 7a. observación argentina.

Summary

The author make a clinical and parasitological study of one new observation of histoplasmosis, parasitical rethiculo-histiocitosis or Darling's disease by *Histoplasma capsulatum* Darling, 1906 in a man 59 year old with pulmonary tuberculouse.

His lesions in the mucouse of the mouth and in the lymph-nodes would healed with sulphadiacine treatment.

It is the 7th. observation in Argentina.

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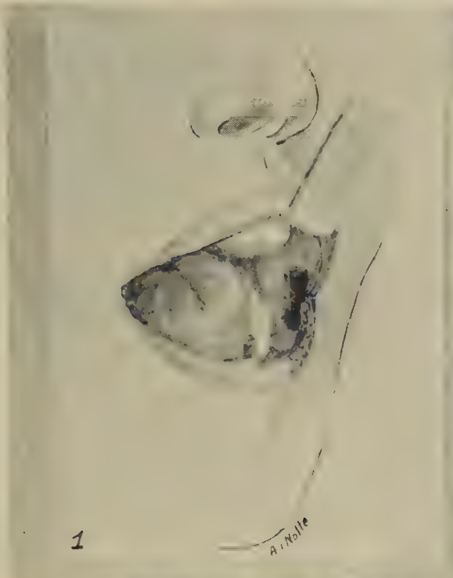
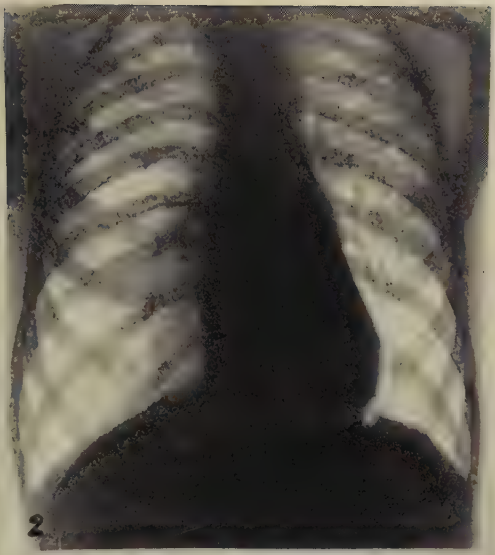
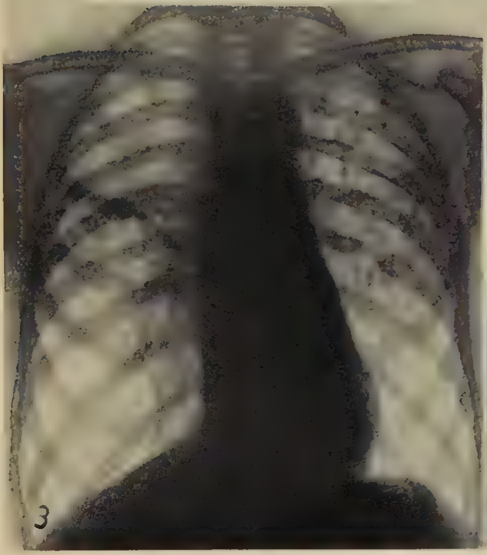






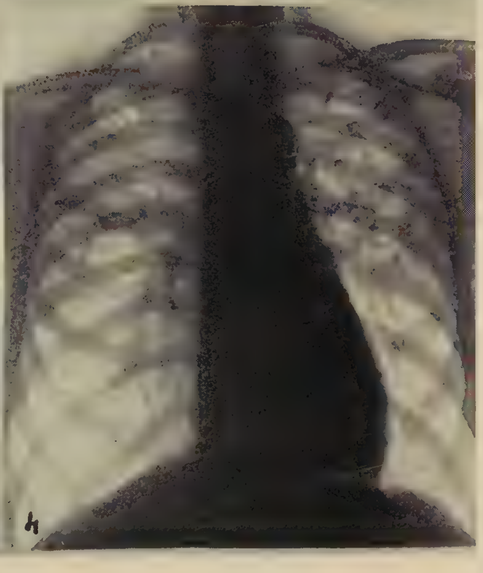
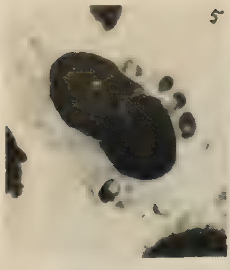


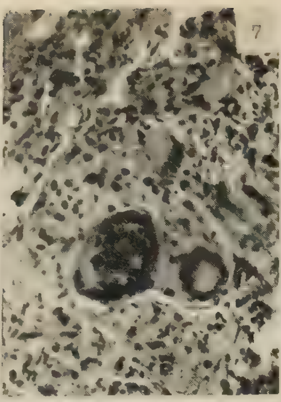
Fig. 1. Dibujo mostrando la situasion y aspecto de las lesiones en la comisura bucal, en la semimucosa labial y en la mucosa bucal vecina la comisura.

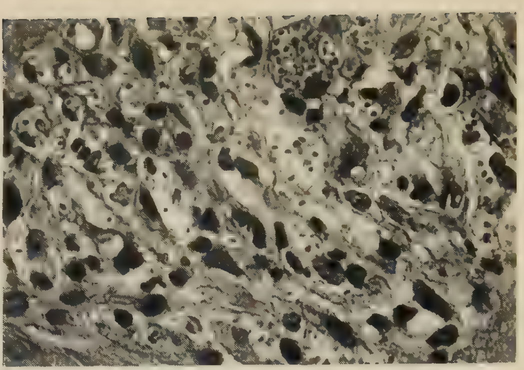
Fig. 2. Radiografía de torax tomada el 22 de Octubre de 1943, mostrando sombras micronodulares en ambos campos pulmonares.

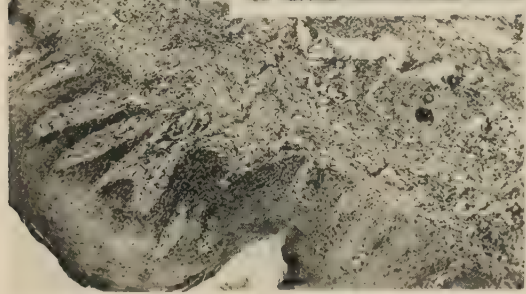
Fig. 3. Radiografía de tórax tomada el 19 de Mayo 1947 que meustra una situación semejante a la anterior.

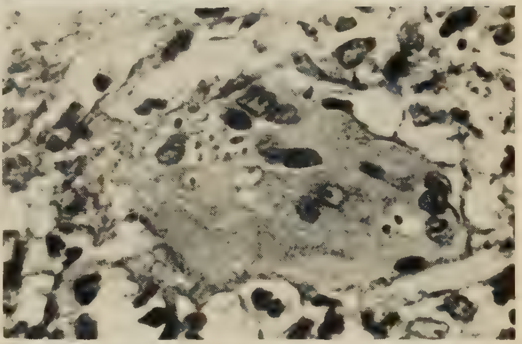
Fig. 4. Radiografía de tórax tomada el 3 de Octubre de 1947, muestra un aumento en el tamaño y diseminación de las sombras micronodulares observadas en ambos campos pulmonares.











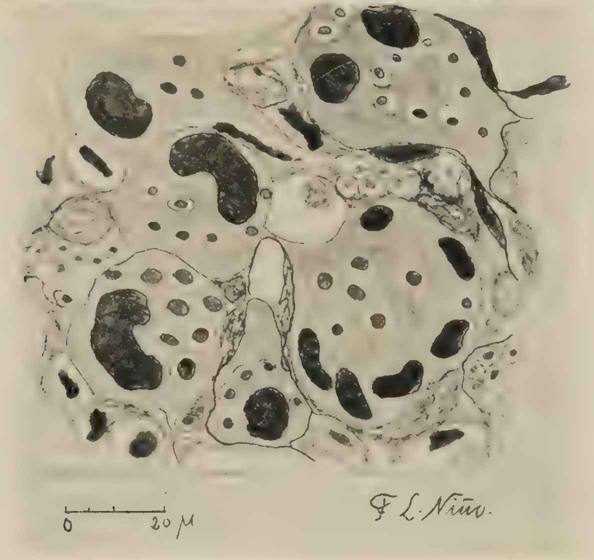


Fig. 5. *Histoplasma capsulatum* en el citoplasma de un gran mononuclear, observado en un frotis por impresión de biopsia de ulceración bucal. (Coloración con May Grünwald-Giemsa. Aumento × 1025).

Fig. 6. Biopsia de ulceración bucal observada con poco aumento. Acantosis e hiperplasia papilomatosa de la mucosa. En el corion, gran proliferación de histiocitos y presencia de granulomas tuberculoides. (Coloración con hematoxilina eosina. Aumento × 70).

Fig. 7. Un campo de la figura anterior tomado con mayor aumento. Granuloma giganto-celular, proliferación histiocitaria y presencia de *Histoplasma capsulatum*. (Aumento × 350).

Fig. 8. Un aspecto de la gran reacción retículo-histiocitaria presentada por el granuloma micósico y presencia de abundantes *Histoplasma capsulatum*. (Coloración con hematocilina férrica-eosina. Aumento × 475).

Fig. 9. Otro aspecto de la retículo-histiocitosis parasitaria mostrando una célula gigante con numerosos parásitos en su citoplasma. (Igual coloración que en la anterior. Aumento × 525).

Fig. 10. Dibujo semi-esquemático de una porción del granuloma micósico tomado con auxílio de la cámara de Edinguer-Zeiss, mostrando una gran cantidad de parásitos en el interior de los elementos retículo-histiocitarios.

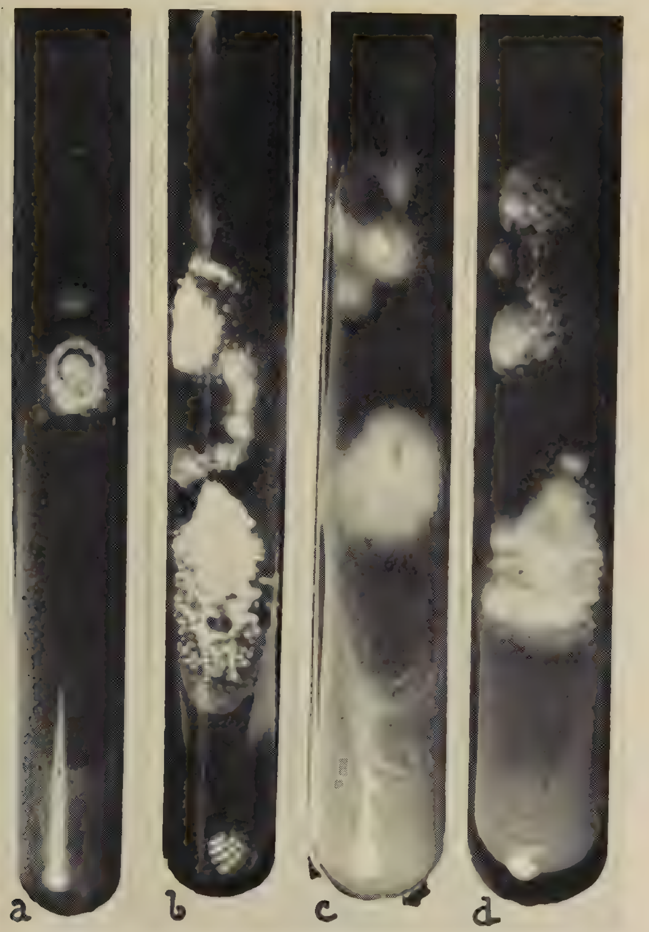


Fig. 1.

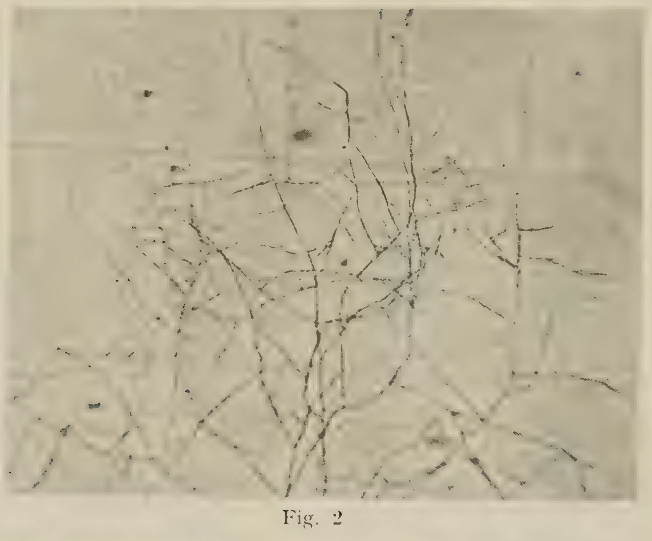
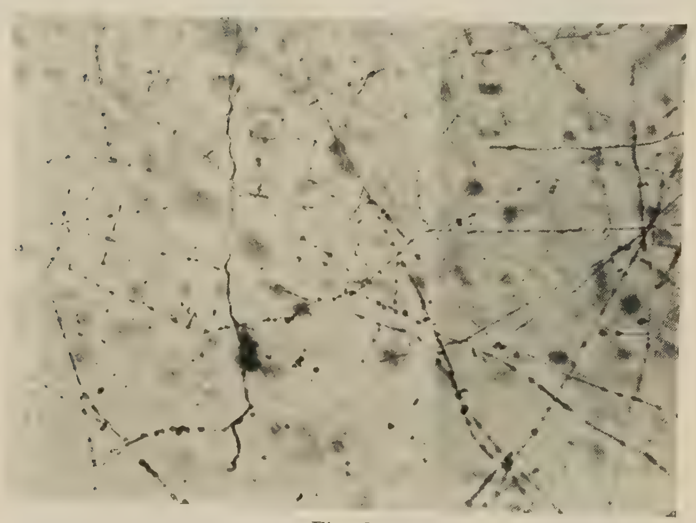


Fig. 2



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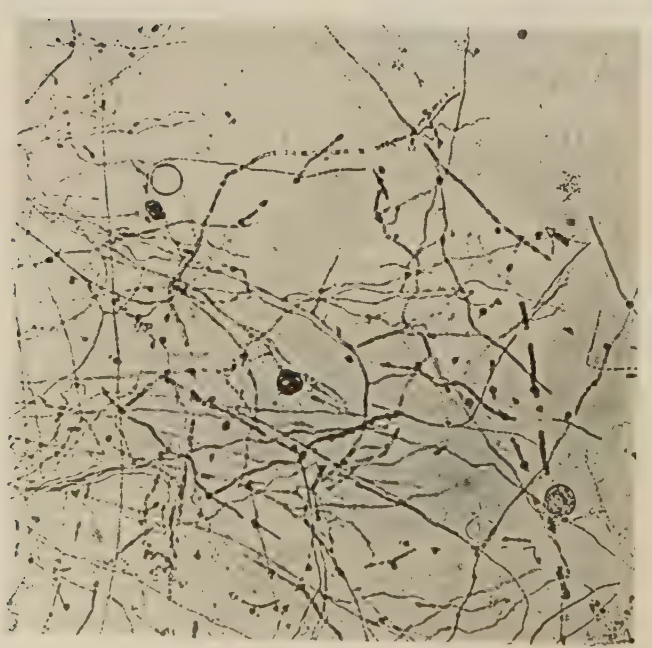


Fig. 4

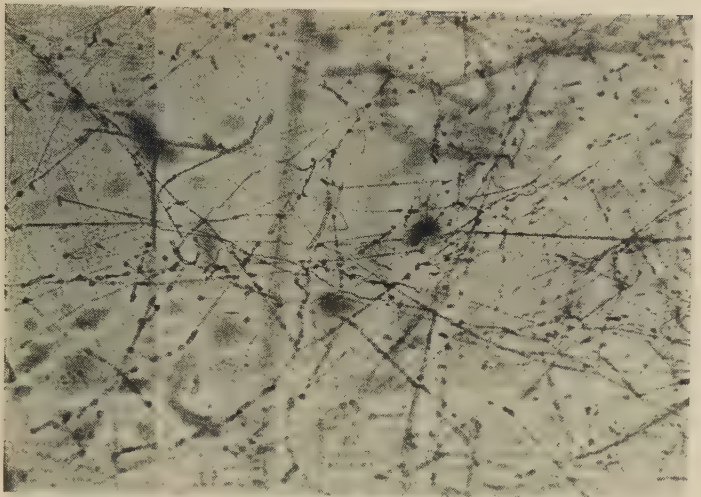


Fig. 5

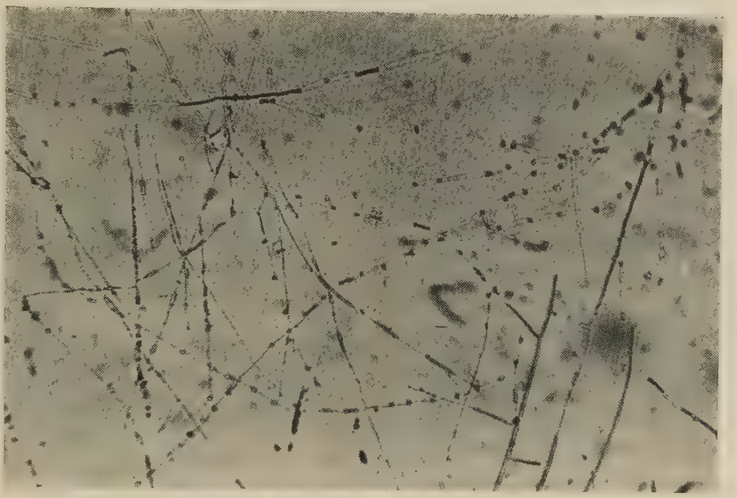
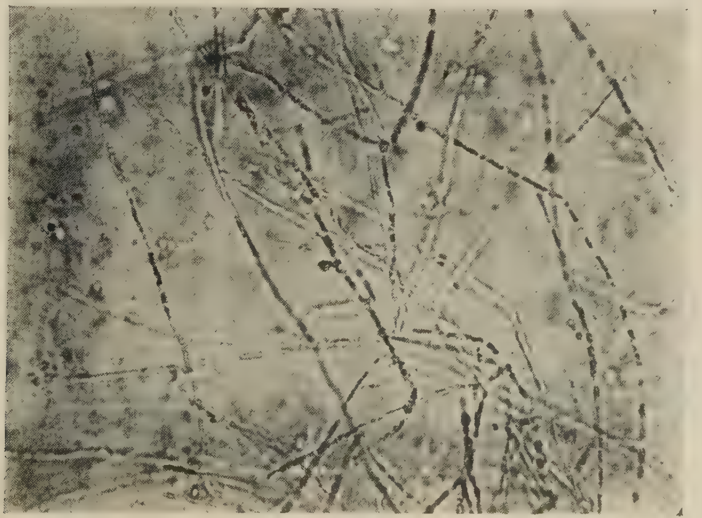


Fig. 6



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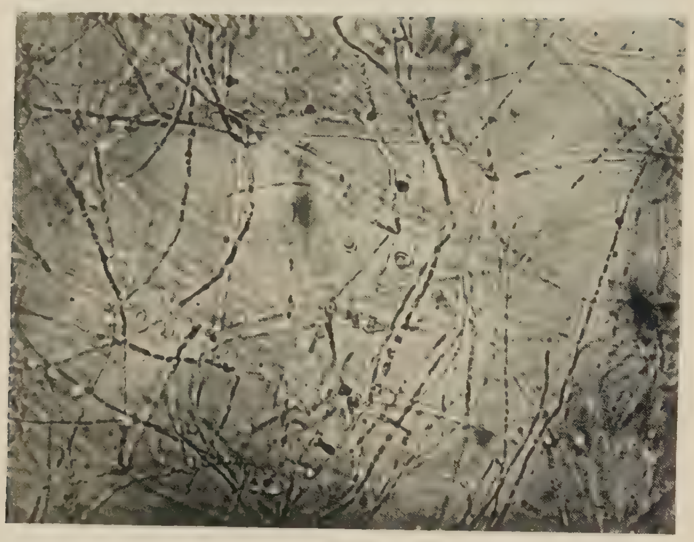

Fig. 8

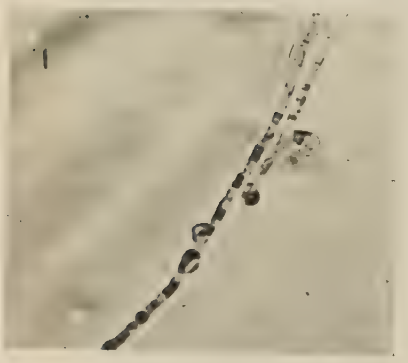

Fig. 9

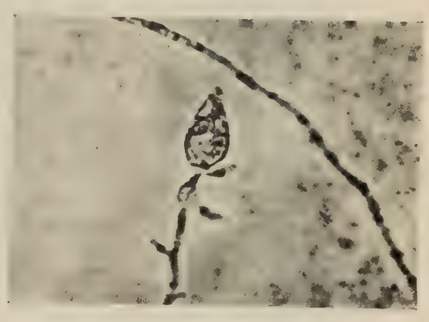

Fig. 10

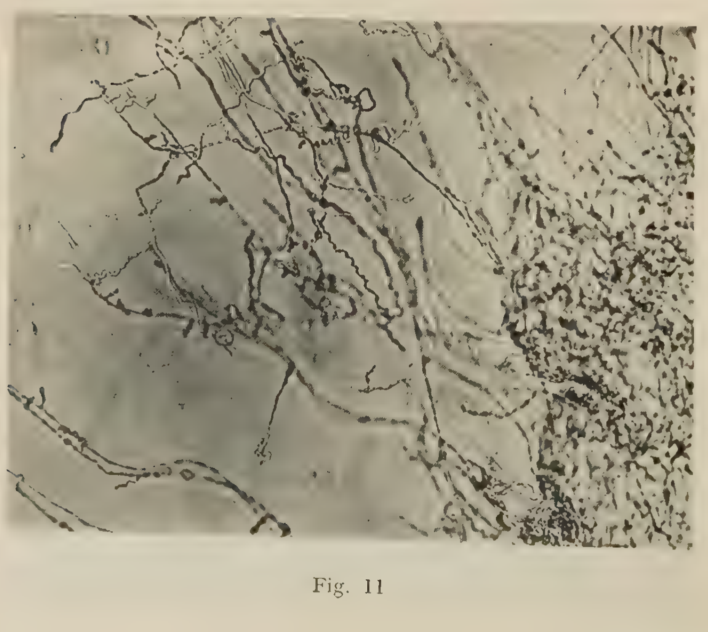

Fig. 11

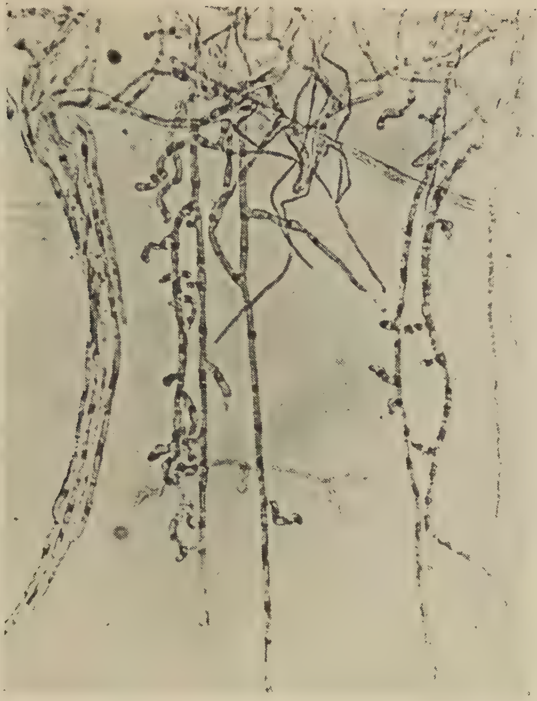

Fig. 12

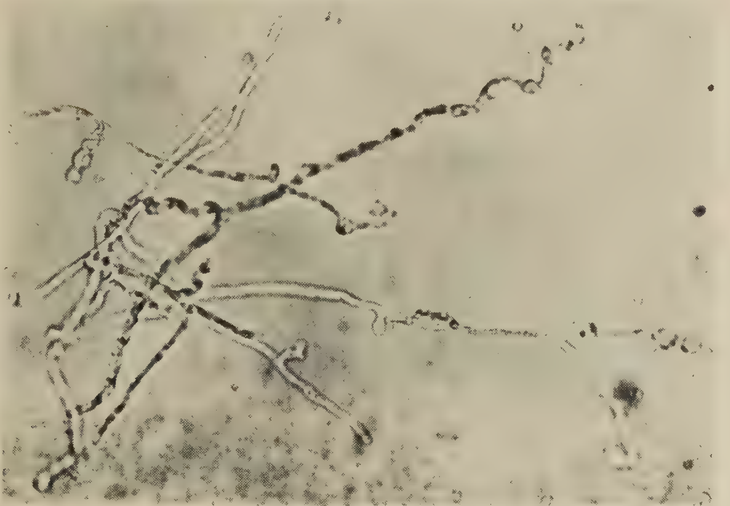

Fig. 13

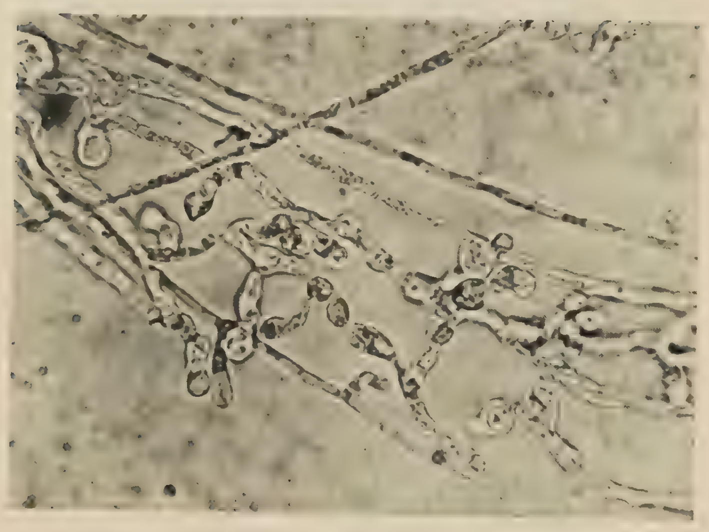

Fig. 14

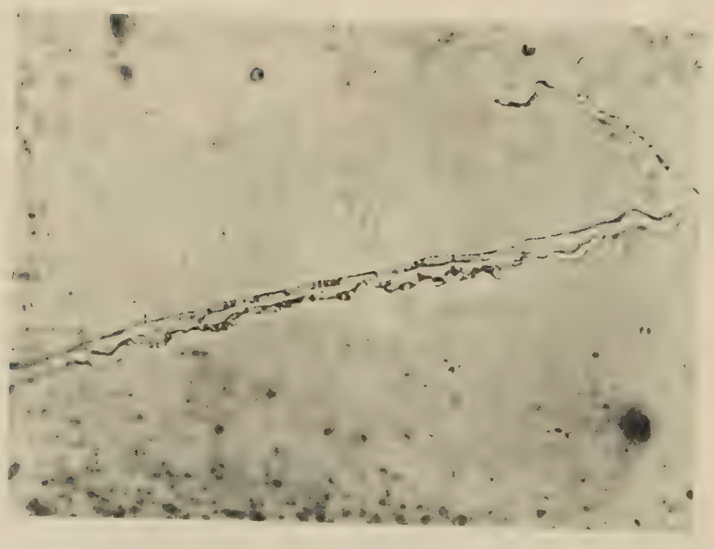

Fig. 15

Researches on the Etiological Agents of the American Blastomycosis.

I. Morphology and Systematic of the Lutz's Disease Agent [1]

by

J. Aroeira Neves and L. Bogliolo.

(Division of the Institute of Hygiene, ex-Ezequiel Dias Institute, and Department of Pathological Anatomy of the Faculty of Medicine at Belo Horizonte, Minas Gerais, Brazil).

(Plate I, II, III and IV, and fig. 1 to 15)

Introduction

The use of the cultural media employed in bacteriology for the study of fungi has been one of the greatest sources of error and confusion for mycologists. So much is this so that we do not think it uncalled-for to quote the opinion of Langeron [11]: "L'esprit de routine à incrusté dans la technique des cultures des champignons quelques prejugés dont il est extremement difficile de la débarrasser. La frequentation préalable des laboratoires de bactériologie est très funeste à ce point de vue pour ceux qui se destinent à la carrière mycologique: il vaut beaucoup mieux avoir des élèves tout à fait ignorants qu'on peut former aux bons principes" (Cf. p. 439).

Among these prejudices he mentions as the most important those caused by the use of synthetic media and of rich media by mycologists. In certain cases the former are not only useful but necessary, *e.g.*, physiological researches, determining the value of a certain foodstuff, fermentations, etc.. But apart from these special cases corresponding to a definite objective their use is not advisable for they do not permit the complete development and that constant morphology of the fungi which is necessary for their classification. The principal objections to rich media are pleomorphism and polimorphism and the appearance of glabrous colonies which may lead to erroneous classification or cause mistaken identification with other fungi in these same media. We have no experience of our own in the use of synthetic media in the cultural study of the causative agent of Lutz' Disease but one of us (J. Aroeira Neves) has had wide experience in the use of rich media especially those of Sabouraud whic are used as a matter of routine for the diagnosis *in vitro* of what has hitherto been called *Paracoccidioides brasiliensis*. We believe that this was done in empirical way both from the macroscopic point of view (culture having a cottony, in aspect ratskin, etc.) and also from the microscopic, as it will be seen later (fig. 15). But such features were not of much use as they are to be found not only in *P. brasiliensis* but also in others as for example in *Blastomyces dermatitidis* and *Coccidioides immitis*, hence the divergences which have led to the confusion existing as to its classification.

It has been mentioned already that one of us has had long experience in the use of rich media for in the period 1915—1946 the specific diagnosis of what was then called *Paracoccidioides brasiliensis* seemed to us somewhat empirical; the cultures were full of aberrant, irregular and polymorphous forms and so did not have that unity of macroscopic and especially of microscopic aspect so necessary for any taxonomic study of the fungus. Consequently we did not consider ourselves able to make a specific diagnosis and so temporarily retained the designation of *Coccidioides immitis* for it. To those who are interested in obtaining further details about synthetic media and those rich in glycidia and in protiolia we recommend the reading of the chapter on that subject in LANGERON's book (15).

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[1] Some of our results concerning the systematic position of the agent of the Lutz's Disease was given by us to *H. Rocha* and *A. Bonfioli* (15) to be included in a communication presented at the 4th. Congress of Ophthalmology at Mar del Plata (Argentina Republic) in December 1948.

From 1946 on, we use a natural medium whose protein, polysaccharide and vitamin contents would promote the fullest development of the so called *Paracoccidioides brasiliensis* and in our opinion the results obtained were satisfactory. We employed the cornmeal agar used by EMMONS (7) in his studies of *Achorion gypseum*. This substrate while stimulating the production of spores, inhibits the formation of non-differentiated cottony mycelium and therefore seems to us the most suitable medium for studying the so-called *Paracoccidioides brasiliensis*. The following is its formula and method of preparation (modified):

Distilled water	3000 mls.
Cornmeal.	125,0 grs.

Heat in a water-bath 1 hour at 60° C.; filter through filter paper; complete to 3000 mls.; add 37,5 grs. of agar; steam for 1¼ hours; filter through absorbent cotton glask, distribute and sterilize in autoclave at 15 lbs. pressure for 30 minutes; place tubes in an inclined position. In order to obtain a more transparent medium free of particles which might beacause of error, the second filtration should be made through filter paper insted of absorbent cotton.

Therefore, as we have found a culture medium in which, as we believe, the morphology of the fungus under considerations is sufficiently constant and characteristic to distinguish it definitely from other fungi, this note will be limited to describing the obtained results and the characteristics of the so-called *P. brasiliensis* in this medium. We have purposely omitted here any discussion of the morphological aspects of *P. brasiliensis* in other culture media and we will not enter into a consideration of problems of a more general nature, as for example the position of the genus *Aleurisma* (which apparently should include *P. brasiliensis*) or the significance of the fructification — *Aleuria* — in the taxonomic diagnosis of certain fungi, etc.. These problems will be ventilated in a later work.

Material used

In our researches we made use of the following strains obtained directly from patients suffering from LUTZ' Disease:

1) Culture isolated from pus from a lymph node of Maria I. at Santa Casa de Misericordia, Belo Horizonte (Profs. Hugo Werneck and Antônio Aleixo's Clinic) in 1915. It is probable that Haberfeld (9) reported this case in his first observation.
2) Culture isolated from pus from a lymph node of Armand M., Dec. 18, 1921.
3) Culture isolated from pus from submaxillary lymph node of Joaquim J. C., Jan. 20, 1922.
4) Culture isolated from pus from a lymph node of José M. S., Oct. 4, 1924.
5) Culture isolated from pus from a lymph node of José M. M. by Dr. Noronha Peres (date unspecified).

6) Culture isolated from abcess of thigh of Waldemar P., (Santa Casa da Misericordia, Belo Horizonte; Prof. Octaviano de Almeida's Clinic), in 1924.
7) Culture isolated from a fragment of the uvula of Antônio P. de S. (Prof. Ildeu Duarte's Clinic) on March 26, 1926.
8) Culture isolated from pus from cervical lymph node of Vinicius P., Sept. 27, 1932.
9) Culture isolated from tumour of the left lower eyelid of Maria C. (Prof. Hilton Rocha's Clinic), Nov. 23, 1932.
10) Culture isolated from a lymph node of João G., June 18, 1938.
11) Culture isolated from cervical lymph node of Theodomiro A. P., Febr. 1, 1939.
12) Culture isolated from a lymph node of Isabel C. by Dr. Paulo Cerqueira Pereira (Viçosa), Sept. 16, 1939.
13) Culture isolated from skin lesion of Isabel C. by Dr. Paulo Cerqueira Pereira (Viçosa), Sept. 16, 1939.
14) Culture isolated from pus from thoracic abcess of Argemiro A., April 22, 1940.
15) Culture nº 1 of Aroeira Neves collection and nº 8 of Bogliolo's collection. Suplied by the Laboratory of Clinical Dermatology of the Faculty of Medicine of S. Paulo (Prof. Aguiar Pupo).
16) Culture nº 2 Aroeira Neves collection, isolated at Juiz de Fóra by Dr. A. C. Pereira from pus from a lymph node of Humberto L.; this strain constitutes the n° 27 of Bogliolo's collection.
17) Culture nº 3 of Aroeira Neves collection and nº 36 of Bogliolo's collection. Obtained from pus from a lymph node of Maria A. I.; isolated Sept. 24, 1945.
18) Culture nº 4 of Aroeira Neves collection and nº 38 of Bogliolo's collection. Obtained of pus from a lymph node of the same patient Maria A. I.; isolated Sept. 28, 1945.
19) Culture nº 5 of Aroeira Neves collection and nº 39 of Bogliolo's collection. Obtained from dental granuloma of the same patient Maria A. I., isolated Oct. 30, 1945.
20) Culture nº 6 of Aroeira Neves collection and nº 39 of Bogliolo's collection. Obtained of pus from a lymph node of the same patient Maria A. I., Oct. 30, 1945.
21) Culture nº 7 of Aroeira Neves collection and nº 42 of Bogliolo's collection; Obtained of material retired by scraping of the gums of Iveta R., Dec. 15, 1945.
22) Culture nº 8 of Aroeira Neves collection and nº 44 of Bogliolo's collection, made from a non-ulcerated lymph node of José A. C., July 14, 1946.
23) Culture nº 9 of Aroeira Neves collection and nº 46 of Bogliolo's collection; made from scrapings of the dental alveolus of José A. C., July 14, 1946, after extraction of second left lower molar tooth with apical granuloma.
24) Culture nº 10 of Aroeira Neves collection. From pus from a non-ulcerated lymph node of José A. C.. Isolated by Dr. Hermeto, July 12, 1946.
25) Culture nº 11 of Aroeira Neves collection and nº 65 of Bogliolo's collection. This sample was given to the latter by Dr. J. Pellegrino and came from Dr. Carlos da Silva Lacaz of the Faculty of Medicine of the University of São Paulo (original label: P. 8 João Rogerio).
26) Culture nº 12 of Aroeira Neves collection and nº 66 of Bogliolo's collection, given to the latter by J. Pellegrino and coming from Dr. Carlos da Silva Lacaz of the Faculty of Medicine of the University of S. Paulo (originally labelled: P. 6 Toulaz).

27) Culture nº 13 of Aroeira Neves collection and nº 67 of Bogliolo's collection, supplied by J. Pellegrino. It came from Dr. Carlos da Silva Lacaz of the Faculty of Medicine of the University of São Paulo (original label: P. 7 L. M.).
28) Culture nº 14 of Aroeira Neves collection and nº 68 of Bogliolo's. Supplied by J. Pellegrino who obtained it from Dr. Carlos da Silva Lacaz of the Faculty of Medicine of the University of S. Paulo (original label: P. 4 Auta M.)
29) Culture nº 15 of Aroeira Neves collection and nº 84 of Bogliolo's from pus from a lymph node of Maria A. S., isolated on May 9, 1947.
30) Culture nº 16 of Aroeira Neves collection and nº 85 of Bogliolo's. From pus from Domingos R. L., isolated on May 23, 1947, during a autopsy made 18 hours after death.
31) Culture nº 17 of Aroeira Neves collection and nº 87 of Bogliolo's. From scrapings from non-liquefied non-suppurating suprahyoidal lymph node of João C. P., isolated on May 28, 1947.
32) Culture nº 18 of Aroeira Neves collection and nº 103 of Bogliolo's. From pus from a lymph node of Emilia M., isolated Aug. 20, 1947.
33) Culture nº 19 of Aroeira Neves collection and nº 104 of Bogliolo's. From scrapings from lingual lesion of Alvino P. de C.; isolated Aug. 29,1947.
34) Culture nº 20 of Aroeira Neves collection and nº 106 of Bogliolo's. From material obtained on Sept. 1, 1947, by puncture of a sterno-cleidomastoidal limph node of Geraldo M..
35) Culture nº 21 of Aroeira Neves collection and nº 112 of Bogliolo's. From pus from a lymph node of above mentioned patient (Geraldo M.). Isolated on Sept. 8, 1947, during a autopsy made 15 hours after death.
36) Culture nº 21 B of Aroeira Neves collection and nº 141 of Bogliolo's. From pus from a sub-mental lymph node of José J. N.; isolated June 1, 1948.
37) Culture nº 21A of Aroeira Neves collection and nº 143 of Bogliolo's. Obtained from pus from a suprahyoidal lymph node of José J. N., June 15, 1948.

The first 14 of the above-mentioned cultures got lost in one way or an other during the period of our researches; the others are to be found in the collection of the Division of the Institute of Hygiene and in the Department of Pathological Anatomy of the Faculty of Medicine.

It is quite possible that two of these strains were studied by REDAELLI and CIFERRI (13) under the name of *Coccidioides histosporocellularis* (Haberfeld) MAGALHÃES (Ceppi da Fonseca ns. 1148 and 1163). Between 1934 and 1935 Prof. O. DE MAGALHÃES, who was then Director of the EZEQUIEL DIAS Institute of Belo Horizonte, sent various strains studied at that Institute to Prof. O. DA FONSECA. These strains were at that time provisionally classified by us as *Coccidioides immitis* until we should come to some definite conclusion as to their classification. It is also well known that in 1932 O. DA FONSECA had adopted the specific name of *Coccidioides histosporocellularis.*

Characterisation of the causal agent of Lutz's disease in cornmeal agar

For this purpose it was used the following technic: spreading of the material (pus, transfered, etc.) not only in the usual way but also taking care to place it on the spot where the medium adjoins the wall of the tube in its upper part. This technic had already been utilised by DE BEURMANN and GOUGEROT (5) and by LANGERON and GUERRA (10) and one of us (2) made use of it with satisfactory results in another mycological study. This technic permits with low magnification to examine directly through and even to follow the development of the colonies of the fungus and to photograph it. It allows, as soon as the organs of reproductions appear, to make a microscopical mycological diagnosis without any necessity of using other artificial means which would probably make classification more difficult.

1) — *Macroscopic features of the causal agent of Lutz's Disease in cornmeal agar.* In this medium the adult colonies of fungus may appear under four chief cultural aspects (Fig. 1, plate XXI): 1) A compact cultural disc surrounded by a less dense mycelial formation weakened and apparently discontinuous in zone. This type which we have called zonated can be well observed by transparency. The margins are indistinct and consisting of sunken hyphae. 2) A central disc which is also compact and surrounded by an efflorescence having the aspect of small aggregations or tufts which remind one of those found in certain gypseous, tricophytons, *e, g. T. lacticolor* and *T. radiolatum.* 3) Filamentous central disc surrounded by thin mycelium which is immersed or prostrate. 4) A mixed type, consisting of combinations of the other three. However other aspects may appear connected with variations in the surroundings. Generally these types of colonies do not spread along the walls of the tube, contrarily to what is generally observed in the cultures of *Blastomyces dermatitidis* and *Coccidioides immitis.* When looked at from the front they vary in colour, according to age, from white to ivory yellow: RIDGWAY (14), Pl. XXX, 21'', O—YY, f., or to light yellow (RIDGWAY, Pl. XXX, 19', YO—Yd). Looked at from the reverse they are cinnamon brown (RIDGWAY, Pl. XV, 15', Y—O, k.) more or less dark according to age.

2) *Microscopic features in cornmeal agar.* In our opinion the microscopic study of the causal agent of Lutz's disease should be carried out on adult fully developed colonies *i.e.* at earliest after the fortieth day of transferred. After this time generally appear numerous aleurias or aleurospores developped from cytoplasmic condensations inside the hyphae, reminding of what happens in most trichophytons and to which SABOURAUD (16) gave the name of protosplamic reserves and later, in 1911, VUILLEMIN (18) distinguished from true conidia because they do not separate immediately from the mycelium by natural processes of dehiscence, but remain attached to the dead and empty hyphe forming, on the surface of the colonies, a kind of farinaceous deposit. In our cultures aleurias (Figs. 3—9, Pl. XXII—XXIV and Pl. I to IV) form the absolutely predominating element and therefore it should serve to characterise these fungus. Although DODGE (6) says that LINK's original figures do not help in the interpretation of the morphological diagnosis of the type species — *Aleurisma sporulosum* — it should be noted that he still maintains the genus *Aleurisma* in his book and even of the species *Blastomyces luteus* as a synonym of *Aleurisma flavissimun.* It should also be mentioned that genus *Aleurisma* has, up to now, been maintained by the same VUILLEMIN (19) and by the different writers on the subject, though opinions are divided as to its systematic position in accordance with the various classifications.

The aleurias of the causal agent of Lutz's disease appears seriate (figs. 3—6, Pl. XXII—XXIII and Pl. I, n° 1, 2; Pl. IV, n° 1, 3, 4, 12, 13) and lateral (figs. 7—10, Pl. XXIII—XXIV and Pl. III, n° 11, 14, 22, Pl. IV, n° 2, 3, 4, 5, 7, 8, 10, 11, 12 and 13) with or without a pedicle at the extremity of the hypha, piriform (Pl. III, n° 14, 22, a. Pl. IV, n° 1, 4, 5, 7, 8, 12) or flattened (fig. 8, a. Pl. III, n° 2, 3, 11), quadrangular (Pl. III, n° 10, 12, 15, 19; Pl. IV, n° 1, 3, 4, 12) or with one of its sides curved (Pl. III, n° 16, 19, a. Pl. IV, n° 3), hyaline. When it is pedicellate, the pedicle may be short or elongated (fig. 8; Pl. III, nº 14 a. Pl. IV, nº 5, 8, 12) and of the same widthe as the hypha to which it adheres; or it may be samller as may be seen by comparing our figs. with figs. 10 and 13, pl. 1, of VUILLEMIN (18), and with the figs. 93, 95 *a* and *b* of VUILLEMIN (19) and with figures 5, 27 to 30 and 32 of COSTANTIN and ROLLAND (4).

Besides aleurias we also meet with lateral, intercalar and terminal chlamydospores (fig. 4 a. Pl. III, nº 17 a. P. IV, nº 17) mor or less abundant in various strains, as well as spiral formations (figs, 11 a. Pl. IV, nº 9).

In 1922 one of us (*Aroeira Neves*) verified the presence of aleurias (Pl. III, fig. 10 to 12, 14 to 16, 19 to 22) in preparations obtained by dissociation of fragments of colonies developed in GORODKOWA's medium and we considered them to be perhaps artificial due to the technic employed. Besides the above we observed in that same year and medium:

a) formations similar to favic candelabra, formed by hyphal tips swolled or not (Pl. III, nº 1 to 4, figs. 12—13, Pl. XXV) and also coremioidal aggregations (Pl. III, nº 8 and fig. 12, Pl. XXV);

b) terminal formations similar to those encountered in *Microsporum lanosum* (Pl. III, nº 5);

c) others comparable to the denticulate apparatus of *M. umbonatum* (Pl. III, nº 5);

d) terminal arborescence comparable to those of *M. villosum* (Pl. III. nº 6, 7 and 8) and other organs which it would be wearisome to mention but which appear in our illustrations;

e) thin, septate hyaline hyphae which are generally straight but sometimes spiral (fig. 11 and Pl. III, nº 13 a. Pl. IV, nº 14), at other times branching Pl. III, nº 24 a. Pl. IV, nº 14), the branches being sometimes joined together (fig. 14 a. Pl. II, nº 2).

Successively, (after 1946), all these forms were found by us, constantly, in cornmeal-agar cultures.

f) As was said above, recent cultures do not supply with sufficiently differentiated forms among which predominate elements which can be taken for chlamydospores (fig. 15 a. Pl. III, nº 18) but which may also evolue in another direction. These features also appear in rich media and are in our opinion the cause of the disagreements as to the systematic classification of the fungus.

Besides it must be pointed out that in the course of time some cultures lose their microscopic characteres by degrees and become pleomorphic; they then do not furnish any safe elements for diagnosis.

3) It will be seen therefore that the cornmeal agar medium used by us gave more uniform results than those obtained by the majority of the authors who employed other media. The morphological aspect obtained in rich media such as potato-destrose-agar and especially in SABOURAUD's medium does not supply any safe indication to the characterisation and differentiation of the causal agent of LUTZ' disease.

To exemplify this assertion it is enough to compare fig. 2 obtained from strain (nº 20) in potato-destrose-agar with fig. 3, the same strain in cornmeal agar, both on the 206th day.

We therefore conclude, as was said in the introduction, that it is inadvisable to use of media rich in polysaccharides for the study of this fungus and possibly of others also; this has also been stated by various AA. besides those we have quoted.

Discussion about the Systematic Position of the Causal Agent of Lutz's disease[1])

CONNANT and HOWELL (3) had reported the characteristic forms of the species, *i.e.* the aleurias, and drew them (although schematically) in Pl. II fig. 19 *a*, *b* and *c*. One of us had in 1922 seen these forms in *Gorodkowa's* medium. We came across them again during the period 1946—1948 in 22 cultures when we had the opportunity of studying almost simultaneously 23 of our 37 strains of the causal agent of LUTZ's disease (called South American Blastomycosis) using cornmeal agar. These formations had been well described by VUILLEMIN (18 a. 19) who called them aleurias for they differ markedly from conidia by not having their own means of dehiscence. CONNANT and HOWELL (3) however maintained for this type of multiplication the term *"formation of lateral conidia by concentration of protoplasm from adjacent cells"*.

REDAELLI and CIFERRI (13), perhaps through having studied the fungus in media rich in glycides and proteins, had no opportunity of observing this type of multiplication, *i.e.* aleurias. They observed the fungus only in its form chlamidospered. The same may be said about the observations of FONSECA (3) and ALMEIDA [1]. As clamidospores is met with in the most dissimilar species of fungus, we believe that it cannot, by itself, as an element that can supply a safe and constant basis for taxonomic purposes (fig. 15).

We therefore agree with CONNANT and HOWELL (3) that the generic name *Blastomyces* is not satisfactory. However, as we have encountered the organs of multiplication which are, in our opinion, characteristic of the genus *Aleurisma*, we think that the correct name of the species causing the LUTZ's disease should be *Aleurisma brasiliensis* and not *Blastomyces brasiliensis* CONNANT and HOWELL, 1942. We are supported also by the opinion of VUILLEMIN (18) who in 1911 found that the genus *Blastomyces* created in 1889 by CONSTANTIN and ROLLAND (4) was a synonym of *Aleurisma* for the typic species — *Blastomyces luteus* — described by them was identical with *Aleurisma flavissimum*, LINK, 1816, CHEVALIER, 1836.

For the same reason the name *Paracoccidioides brasiliensis* (SPLENDORE) ALMEIDA, 1930, and *Lutziomyces histosporocellularis* (HABERFELD) FONSECA, 1939, should be abandoned.

In 1911 VUILLEMIN (18) distinguished two groups of aleurosporates, one corresponding to the sporotrichates and the other to the sporophorates. In the first group he placed the family of the *Aleurismaceae* and in the second the *Monostoporaceae*. But, in 1931, he included the genus *Aleurisma* in the order of the *Arthrosporates*. In 1945 LANGERON (11) adapted VUILLEMIN's (18) classification to MASON's (12) and isolated the family *Aleurismaceae* (without distinct sporophores) with *Aleurisma* LINK, 1809 as the typical genus. This family was divided into two sub-families, *Aleurismatae* whose aleurias are monocellular and *Blastotrichatae* whose aleurias seem to be bicellular or pluricellular; in this last group he placed the dermatomycetes distributed among various genera.

The differentiation of the apparatus of reproduction in these two subfamilies, that is the condensation of cytoplasm in certain segments of the hypha and consequent degeneration or death in others (similar to the characteristics observed in our cultures), is further justification for placing the causal agent of LUTZ's disease in the genus *Aleurisma*.

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1) We have been able until present to examine 4 strains of the causal agent of the so-called North American Blastomycosis (*Blastomyces dermatitidis*, CONNANT and HOWELL, 1942) and in a future paper we shall deal with its characterisation.

We may add that the sub-division into two sub-families seem logical to us and seems to indicate a possible relationship of the species *Aleurisma brasiliensis* with certain other species included among the *Blastotrichatae*. In classifying some of these fungi, we have purposely avoided the problem of the inclusion of the forms present in their parasitic phase. In spite of the fact that their recognition is important and that their characteristic morphology is sufficient for a generic and specific diagnosis in certain diseases and in some cases they very rarely supply us with a firm basis for taxonomic purposes.

Summary and conclusion

We have been able to establish typical characteristics of the species, in artifical culture media, of the fungus causing LUTZ's disease. Our work is based on the macroscopic and microscopic study of 37 strains of the fungus and chiefly on the almost simultaneous study of 23 cultures (15 to 37) in various culture media, among them cornmeal agar which in our opinion is a medium indispensable for differentiating and for the classification of the fungus from other agents which produce syndromes of a like nature.

In addition, a revision of the literature and a comparison of the forms presented by the causal agent of LUTZ' disease with the illustrations in the works of VUILLEMIN (18) and of CONSTANTIN and ROLLAND (4) have led us to include the fungus in question in the genus *Aleurisma* LINK, 1809. We were however unable to consult LINK's original work which is referred to and discussed by the quoted authors and by others. Therefore admitting the validity of the genus *Aleurisma* we believe that the name of the etiological agent of LUTZ's disease should be changed to *Aleurisma brasiliensis* (SPLENDORE), *nobis*, being on the microscopic study of the fungus in cornmeal agar and completed by the forms found in media rich in glycides.

Its specific diagnosis are the following: *Aleurisma brasiliensis: In pauperis substratibus gignuntur hyphis mycelicis hyalini, septati, moderate ramose cum aleuriis simplicibus, seriatis, apicalibus vel lateralibus, hyalinis, conspicuis.*

Chlamydosporis intercalaribus, apicalibus vel lateralibus in substratis carbohydratis plenis.

Oidium brasiliensis SPLENDORE, 1912 (cf. FLORIANO DE ALMEIDA — Micologia Médica, 1939, p. 424 — São Paulo — Brazil);

Blastosporidium Schooi HARTMANN, 1912 (Nederl. Tijdschr. v. Geneesk., **56** (**2**): 105—116, 1 pl.);

Zymonema brasiliense SPLENDORE, 1912 (Bull. Soc. pathol. exot., **5** (**5**): 313—319);

Zymonema brasiliense SPLENDORE, 1912, Volume in onore del Prof. ANGELO CELLI, Roma, Tipogr. Nazionale.

Mycoderma histosporocellulare BRUMPT, 1912, NANNIZZI, 1934, CIFERRI & REDAELLI, 1936, Bollettino dell'Instituto Sieroterapico Milanese, **15** (**2**): 97—102.

Zymonema histosporocellularis HABERFELD, 1919 (Tèse, 108 pp., 21 figs., São Paulo — Brazil);

Mycoderma histosporocellularis NEVEU — LEMAIRE, 1921 (Précis Parasitol. Humaine, p. 68);

Monilia brasiliensis VUILLEMIN, 1922 (NANNIZZI, in C. POLLACCI, Trattato di Micopat. Umana, p. 82, 1935);

Mycoderma brasiliense BRUMPT, 1927 (in Précis Parasitol., 1936, vol. II, p. 2030);

Coccidioides brasiliensis (SPLENDORE, 1912) ALMEIDA, 1929 (Ann. da Fac. de Med. de São Paulo, vol. IV, pp. 91—98);

Paracoccidioides brasiliensis (Splendore, 1912) Almeida, 1930 (C. R. Soc. Biol., 105, pp. 115—316);

Coccidioides histosporocellularis Fonseca, 1932 (Zeissler, Arch. Derm. Syphilol, 15: 171—185);

Paracoccidioides cerebriformis Moore, 1935 (Rev. Biol. a. Hyg., VI, nº 2, pp. 148—154, pls. II a IX);

Protomyces Faverae Dodge, 1935, *apud* Redaelli & Ciferri, 1942. Trattato di Micopatologia umana diretto dal Prof. G. Pollacci, vol. V, p. 269, Sansoni, Firenze.

Paracoccidioides tenuis Moore, 1938 (in F. Almeida, Micologia Médica, 1939, p. 437);

Lutziomyces histosporocellularis (Haberfeld, 1919) Fonseca, 1939 (in O. da Fonseca, filho — Parasitol. Médica, vol. I, 1943, p. 245);

Blastomyces brasiliensis Connant & Howell, 1942 (Jour. Invest. Dermat., 5: 353—370);

Coccidoides immitis Stiles, in Rixford & Gilchrist, 1896, seconding to north-american, south-american and europeans AA., to 1930.

NOTE. — We have to thank Dr. Waldemar Versiani for the translation into latin of the specific diagnosis of the fungus.

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Explanation of the figures

Fig. 1. Macroscopic aspects of the causing agent of Lutz's Disease on cornmeal agar. *a*) Strain n⁰ 35, 115 days old: zonated type; *b*) Strain n⁰ 33, 115 days old; *c*) Strain n⁰ 20, 115 days old; *d*) Strain n⁰ 36, 115 days old.

Fig. 2. Strain n⁰ 20, potato-agar destrose, 206 days old. Hiphae straight or branched. Microphoto obtained directly through the glass of culture-tube. × 150.

Fig. 3. The same strain (n⁰ 20) of the above fig. 2. Culture on cornmeal agar. Fruictified hyphae. Microphoto obtained directly through the glass of the culture-tube. × 150.

Fig. 4. Strain n⁰ 24, cornmeal agar, 76 days old. Numerous aleuriae and four chlamydospores. Microphoto obtained directly through the glass of culture-tube. × 150.

Fig. 5. Strain n⁰ 34, cornmeal agar, 176 days old. Aleuriae. Microphoto obtained directly through the glass of culture-tube. × 150.

Fig. 6. Strain n⁰ 24, cornmeal agar, 120 days old. Fructified hyphae. Microphoto obtained directly through the glass of the tube. Compare with fig. n⁰ 2 of the Plate I. × 150.

Fig. 7. Strain n⁰ 22, cornmeal agar, 73 days old. Dissociated with lactophenol of Amman between cover glass and slide. × 800.

Fig. 8. Strain n⁰ 22, cornmeal agar, 73 days old. Dissociated with lactophenol between cover glass and slide. Sessile aleuriae; a pediculate and squashed aleuria. × 700.

Fig. 9. Strain n⁰ 20, cornmeal agar, 76 days old. Dissociated with lactophenol between cover glass and slide. Lateral and terminal simple aleuriae. × 800.

Fig. 10. Strain n⁰ 23, cornmeal agar, 76 days old. Dissociated in lactophenol, between cover glass and slide. Five lateral aleuriae and a terminal chlamydospore. × 700.

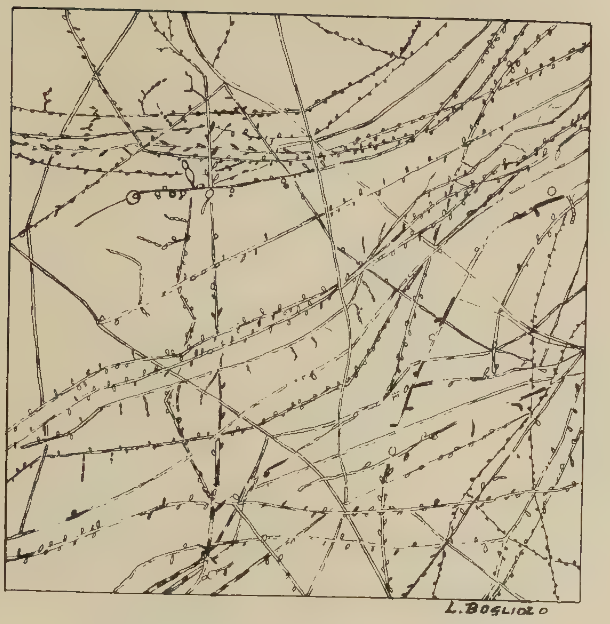
Fig. 11. Strain no 20, cornmeal agar, 76 days old. Dissociated in lactophenol, between cover glass and slide. Soft spiral hyphae. × 150.

Fig. 12. Strain n⁰ 20, cornmeal agar, 76 days old. Dissociated in lactophenol between cover glass and slide. Branched hyphae, candelabrum, coremia-like formation. Lateral aleuriae. × 700.

Fig. 13. Strain n⁰ 23, cornmeal agar, 26 days old. Dissociated in lactophenol, between cover glass and slide. Candelabrum resembling to those of *Achorion Schoenleinii*. Mycelial branch dichotomized with one of the branches. × 700. Compare with figs. 2 to 9 of the Pl. I.

Fig. 14. Strain n⁰ 23, cornmeal agar, 26 days old. Dissociated in lactophenol between cover glass and slide. Bifurcated hyphae (one almost straight, the other forming a spiral); anastomosis between the hyphae and probably soldering of the terminal extremitis of the spiralated hyphae in the straight branch. × 700.

Fig. 15. Strain n⁰ 23, cornmeal agar, 26 days old. Dissociated in lactophenol, between cover glass and slide. Inespecific formations making impossible any mycologic diagnose (see in the text). × 1.200.



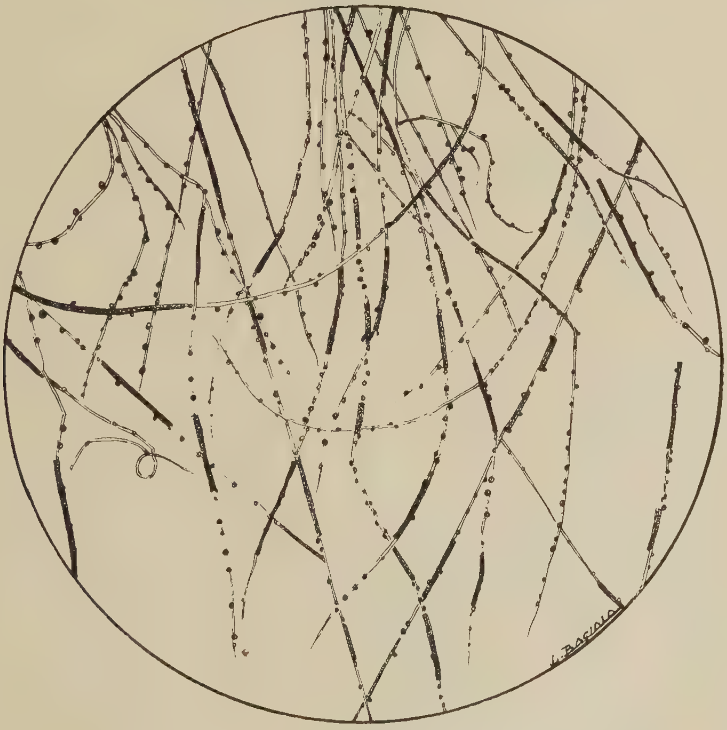


PLATE I

No 1. Strain n⁰ 18, on cornmeal agar, 88 days old. Pictures obtained directly through the glass of the tube. × 145.

No 2. Strain n⁰ 24, on cornmeal agar, 110 days old. Picture obtained directly through the glass of the tube. × 96.

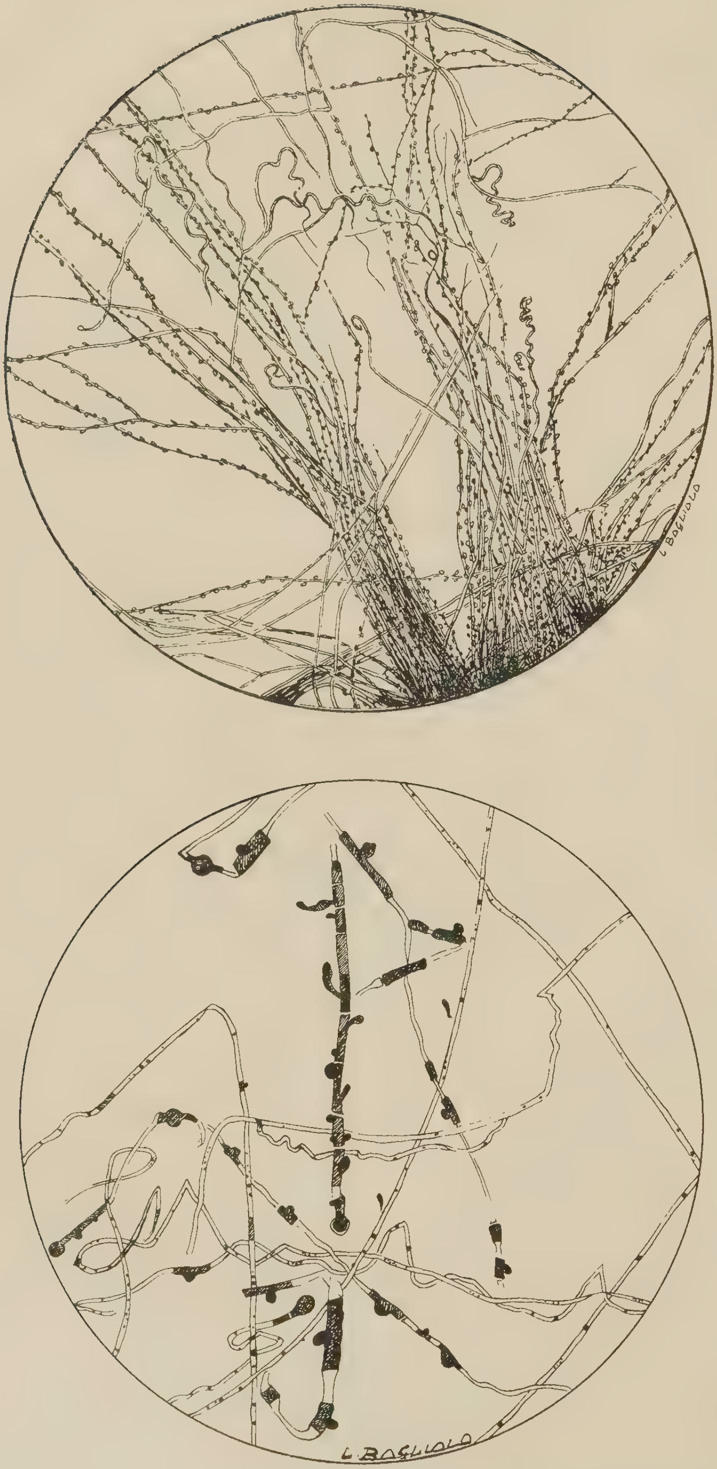


PLATE II

No 1. Strain n^0 28, on cornmeal agar, 110 days old. Coremia-like formations, fruitified. Spirals. Picture obtained directly through the glass of the tube. × 96.

No 2. Strain n^0 20, on cornmeal agar, 76 days old. Dissociated in lactophenol. Various types of aleuriae and mycelial formations. × 700.

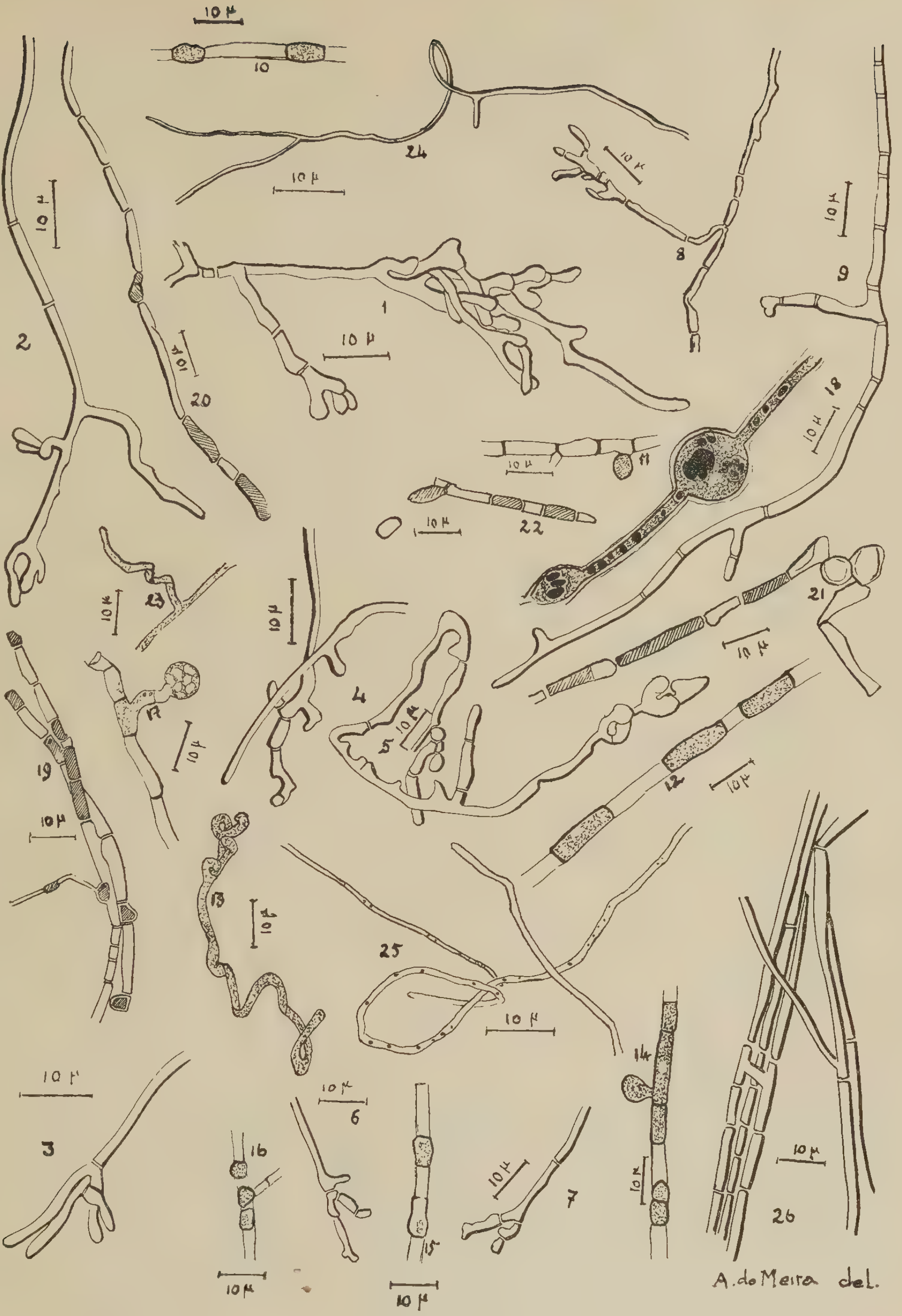

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PLATE III

ESPLANATION OF THE FIGURES

Ns. 1, 2, 3. Candelabrum. No. 4. Mycelial termination in the form of candelabrum pierced by others similar to those found in *Achorion schoenleinii*. 5 month old culture. No. 5. Fragment of a typical hypha with terminal formations-like those described by Sabouraud in *Microsporum lanosum* and formations which remind one of the denticulate apparatus of the *M. umbonatum* of the same author. Ns. 6, 7, 8. Terminal mycelial arborescence comparable to those of *M. villosum*. No. 9. Micelial filament with a lateral branch, reminding one of those met with in filaments of *Achorion schoenleinii*. Ns. 10, 12, 14, 15, 16. Aleurias. In 14: sprouting of an aleuriform formation. No 11. Lateral aleuria. No 13. Mycelial filaments showing a beginning of spirals. No 17. Mycelial differentiation with lateral dilatation. No 18. Mycelial growth reserve. Ns. 19, 20, 21, 22. Aleurias. No 23. Spiral ramification. Ns. 24, 25. Very fine, fragile mycelial filaments. Branching in n^0 24. No 26. Micelial filaments showing a spirale.

NOTE. Figures obtained from culture n^0 2 in Gorodkowa's medium, slightly dissociated in Amman's lactophenol between a slide and coverslip. All the figures were obtained from 5 month old culture, except n^0 18 which was from a 9 days old culture (compare with the fig. 15).

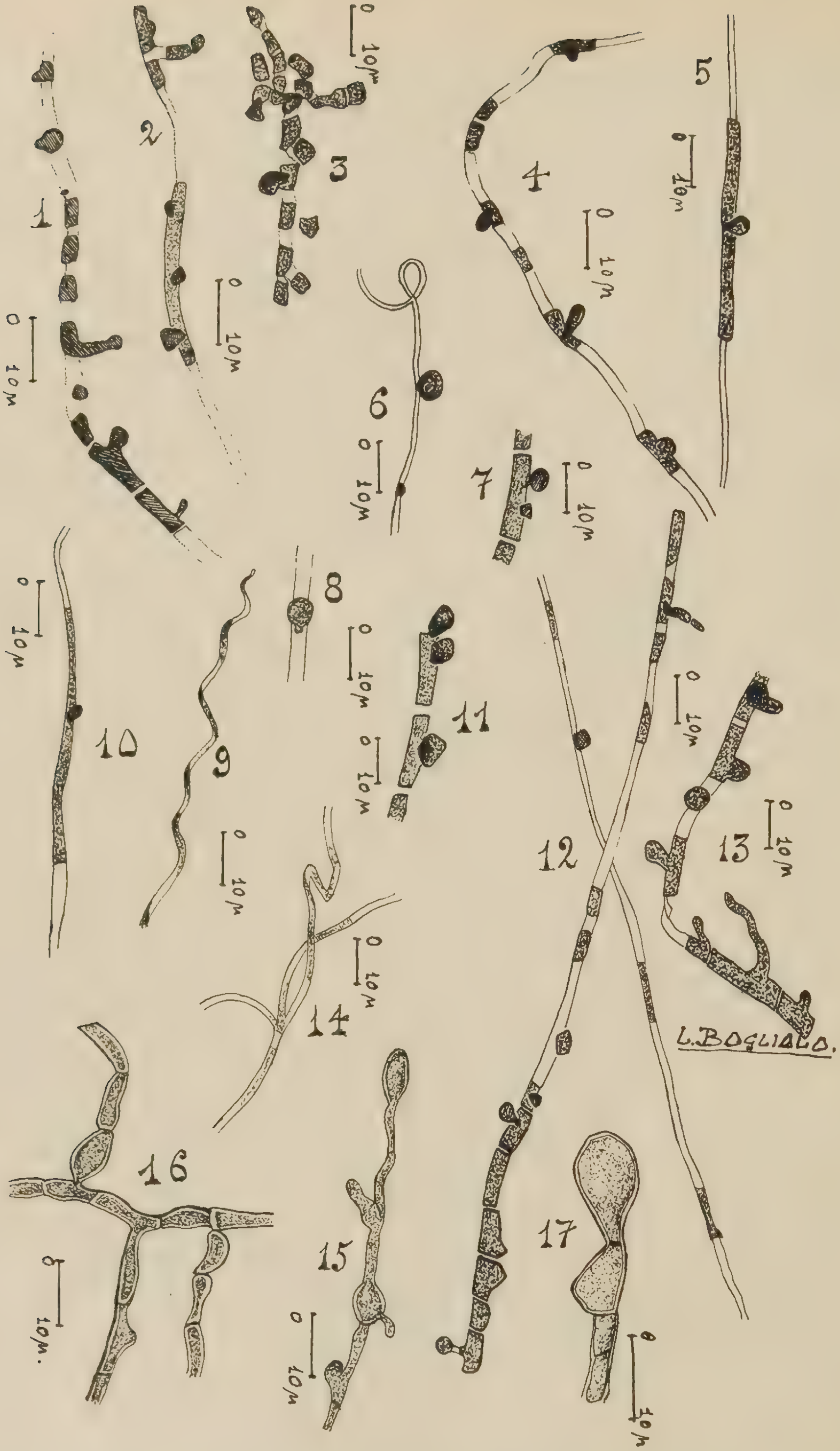

PLATE IV

Ns. 1, 2, 3, 4, 5, 7, 10, 11, 12, 13. Various types of aleurias.
No 6. Aleuria of anomalous type?
No 9. Spiral termination of hypha.
No 14. Type of mycelial branching.
Ns. 15, 16, 17. Indefinable forms registered by most observers.

NOTE. Figs. 1 to 14 were obtained from strain nº 20, from a 76-days old culture in cornmeal agar, dissociated in lactophenol between slide and coverslip. Ns. 15, 16, 17, strain nº 34, from an 80 days old culture in cornmeal agar, dissociated in lactophenol between slide and coverslip.

Alguns fungos de fumagina de Pernambuco

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O trabalho em tela compreende uma ampla investigação ácerca dos fungos de fumagina, visando o esclarecimento de sua posição taxonomica em Pernambuco.

Na realidade foram grandes os tropeços com que lutamos, dada a diversidade de especies de fungos e suas relações simbionticas com insetos, exigindo-nos um esforço imenso na elucidação das questões a que nos propunhamos conhecer.

Em trabalho anterior, ¹) apresentado á Sociedade de Biologia de Pernambuco, oferecemos uma lista completa dos fungos que identificamos como agentes de fumagina. Agora, limitamo-nos á descrição das especies novas e de dois novos generos, que havemos criado.

De modo geral, ficou ressaltada a estreita associação entre diferentes especies de fungos e o inseto *Orthezia insignis* Douglas no estabelecimento das fumaginas. Figs. 1, 2, 3, 4 e 5 (Pl. XXVII—XXVIII).

Entre os *Capnodiaceas* e *Meliolaceas* que apreciamos, incluimos tambem alguns fungos *Microthyriales,* que originando pontuações negras sôbre os orgãos vegetativos das plantas, são tidos, igualmente, como causa de fumagina.

O nosso reconhecimento é tornado expresso ao Agro. ALBINO FERNANDES VITAL, ao Prof. DARDANO DE ANDRADE LIMA e outros colegas que nos ajudaram de alguma fórma na marcha da pesquisa que este trabalho condensa.

Trichomerium abhorrentis n.sp.

Capnodiaceas

Micelio negro, epifilo, membranoso-crustaceo, estendendo-se por toda a pagina das folhas e ás vezes pelo peciolo e caule; hifas dematioides, fuscas, intercruzadas em todas as direções, sem hifopodios; setas ausentes; celulas hifais bojudas ou alongadas, de 12,5-20 × 5-7,5 u.

Peritecios negros, astomos, globosos, de estrutura pesudo-parenquimatica e consistencia membranosa, com celulas parietais de 5—12,5 u de diametro; setosos, variando o comprimento das setas de 95—150 u; esparsos ou gregarios, e assentes sôbre subiculo, com as dimensões de 125—215,5 u.

Ascos elipticos, apedicelados, aparafisados, octosporos, polisticos, agrupados á maneira de umbela, 50—65 × 22,5—27,5 u.

Ascosporos hialofragmos, fusoides, polisticos, 27,5—35 × 7,5—10 u.

Picnidios ausentes.

Triposporium presente.

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¹) Lista de fungos de fumagina e aliados.
Prof. A. Chaves Batista e Albino Vital. ANAIS da Soc. de Biologia de Pernambuco, T.VIII, no. 1, 1948.

Isolado sôbre erva de rato — *Palicourea nicotianaefolia* C. e Schl. em associação com *Orthezia insignis* Douglas.

O material tipo tem o no 1262, no herbario da Secção de Fitopatologia, do I.P.A., havendo sido colhido em Apipucos, Recife p.m. 960.

S/ grão de galo-*Cordia nodosa*, mat. no 1275

Mycelium nigrum, epiphyllum, membranoso-crustaceum, ex hyphis articulatisque dematioidis; cellula 12,5—20 × 5—7,5 u.

Perithecia nigra, astoma, globosa, membranosa, pseudoparenchymatica, 125—215,5 u, sparsa vel gregaria; parietibus ex cellulis globosis 5—12,5 u diam. Setis perithecialis simplicis, 95—150 u longae. Ascis ellipticis, apedicellatis aparaphysatis, octosporis, polystichis, 50—65 × 22,5—27,5 u.

Ascosporis pluriseptatis, hyalinis, fusoidis, 27,5—35 × 7,5—10 u. Spermogonia ignota. Triposporium presentis.

Ad folia viva *Palicourea nicotianae folia* C. e Schl. cum *Orthezia insignis* D.

Typus 1262 in Secção de Fitopatologia-Instituto de Pesquisas Agronômicas-Pernambuco-Brasil.

Trichomerium crotoni n.sp.

Capnodiaceas.

Micelio epifilo, crustoso, negro, recobrindo toda a superficie da folha. Hifas constritas, dematioides, intercruzadas em todas as direções, com celulas de 8,75—15 × 3,75—7,5 u. Ausencia completa de hifopodios e de setas.

Peritecios negros, globosos ou piriformes, 50—65 u, sesseis, setosos, esparsos no micelio e de situação superficial, Figs. 6 e 7 (Pl. XXVIII—XXIX).

Paredes periteciais formadas por celulas dematioides, aparentemente poligonais, mas de hifa meridiana constituida de celulas globosas; essas celulas parietais têm o diametro de 5—10 u; — consistencia membranosa; ostiolo não definido.

Ascos clavados a elipticos, apedicelados, aparafisados, octosporos, polisticos, de disposição basal umbelada, 37,5—50 × 17,5—25 u. Fig. 8. (Pl. XXIX).

Ascosporos hialofragmos, de x septos, não constritos e cilindrico-fusoides, 19,8—28 × 6,63 — 7,5 u.

Picnidios gregarios, alongados, bojudos a cilindricos, tendo o bojo localizado numa região equidistante dos extremos. A sua altura é de 234—325 u; o pescoço propriamente dito varia de 78—143 × 10—12,5 u, enquanto a porção dilatada tem o diametro de 20—30 u. Fig. 9. (Pl. XXIX).

Paredes do picnidio mais ou menos laxas, de consistencia membranosa; ostiolo não fibrilado. Picnidiosporos globosos ou bacilares, amerosporos, hialinos, expulsos em cirro e aglutinados em bola em torno do ostiolo, 3,75—5 × 2—2,5 u.

Isolado sobre *Croton sp.* no Monteiro-Recife.

Material herb. 1164 e 1165 p.m. 774, 775, 776, 777, 778, 779, 780, 781, 782, 783, 784 e 785.

Ob. em Simbiose com *Orthezia insignis* Douglas.

Mycelium nigrum, epiphyllum, pelliculosum; Hyphis septatis, torulosis, constrictis, ex cellula 8,75—15 × 3,75—7,5 u; hyphopodia absentis.

Pycnidia gregaria, nigra, numerosa, collo longo 78—143 u. Ostiolum non fimbriatum. Sporidia hyalina, oblonga vel bacillar, in substantia gelatinosa agglutinata.

Peritheciis globosis, 50—65 u, membranaceis; parietibus ex cellulis globosis, 5—10 u.

Ascis octosporis, elliptico-clavatis, non pedicellatis, — 37—5—50 × 17,5—25 u; paraphysibus nullis. Ascosporis pluri septatis, hyalinis, fusoideo- cylindraceis, 19,8—28 × 3,75—6,6 u.

In foliis vivis *Croton sp* et in symbiose *Orthezia insignis* D.-typus 1164 in herbarium Secção de Fitopatologia-Instituto de Pesquisas Agronomicas.

Trichomerium psidii n. sp.

Capnodiaceas

Micelio dematioide, negro, peliculoso, sem hifopodios, sem setas, mas, possuindo triposporium, facilmente destacavel; hifas torulosas, fuscas, distribuidas epifilamente em linhas retas e em todas as direções, de maneira a revestir por inteiro a superficie das folhas.

Celulas hifais de 12,5—17,5 × 3,75—10 u.

Essa coberta do fungo se processa numa simbiose franca com o inseto *Orthezia insignis* Douglas.

Peritecios negros, globosos, papilados, astomos, membranosos e dotados de setas; estrutura das paredes de natureza pletenquimatica, constituida por celulas de 7,8—15,6 u. O seu diametro está entre — 125—235 u. Setas de 70—125 × 5—7,5 u. Fig. 10. (Pl. XXIX).

Ascos elipticos, octosporos, apedicelados, aparafisados, de apice rotundo, 40—57,5 × 15—20 u.

Ascosporos fusoides, hialofragmos, polisticos, triseptados em geral, de modo a se apresentarem quadriloculares. 17,5—22,5 × 5—6,5 u.

Picnidios de estrutura tambem pletenquimatica, ramificados ou não, isolados ou grupados, e de forma semelhante á de garrafa; o bojo mede de 30—56 u de diametro, e o pescoço, que é alongado, varia de 120—225 × 8,4—14 u, mostrando a extremidade livre constituida por fibrilas. Fig. 11. (Pl. XXX).

Conidios liberados em cirro, elipticos, unicelulares e hialinos, 2,5—3,75 × 1,25u.

Isolado sôbre *Psidium guayava* L.-Casa Forte. - Recife

Mat. tipo herb. sob no 1153 e p. m. 724 e 729.

Tambem isolado sobre: *Cassia rosea* — *C. nodosa* — 1184, *Psidium guayava*, 1188, Apipucos, em simbiose com *Orthezia insignis* Douglas.

S/ *Anacardium occidentale*, 1189, Apipucos, em simbiose com *Orthezia*. S/ *Mangifera indica L.* c/ *Vinsonia stellifera* Westwood e *Pseudaonidia trilobitiformis* Green 1191.

S/ *Psidium araçá Raddi* — 1177.

S/ camboatan — *Cupania sp.* 1250 — em associação com *Orthezia* in *signis* D. S/ Caferana — *Tachia guyanensis* — Apipucos. Mat. no 1267, sem inseto simbiontico. S/ *Phylodendron imbé.* mat, no 1268, sem inseto simbiontico. S/ *Xylopia frutescens* — imbira vermelha — 1279. S/ Amoreira — *Morus alba*, mat. no 1283, c/ *Orthezia*. S/ feto — *Adianthum* sp., com *Orthezia*, 1284. S/ barbasco-*Euphorbia phosphorea.* Mez, 449.

Mycelium nigrum, epiphyllum, effusum, pelliculosum, facile destacabile. Hyphis septatis, constrictis, articulatisque torulosis, brunneis, rectis dispositis, ex cellula 12,5—17,5 × 3,75—10 u.

Peritheciis sessilibus, astomis, fusco-nigris, globosis, 125—235 u in diam., setulatis, 70—125 × 5—7,5 u; parietibus ex cellulis 7,8—15,6 u. Ascis octosporis, elliptico-elongatis, 40—57,5 × 15—20 u, apedicellatis aparaphysatis. Ascosporis fusoideis, hyalinis, polystichis, qudriseptatis, 17,5—22,5 × 5—6,5 u.

Spermogonia nigra, 30—56 u diam, numerosa, collo longo, apicem —

fimbriato, 120—225 u longa, 8,4—14 u diam. Sporidia hyalina, 2,5—3,75 × 1,25 u in substantia gelatinosa agglutinata, elliptica.

In folliis vivis *Psidium guayava L.* Typus 1184 in herbarium Secção de Fitopatologia, Instituto de Pesquisas Agronomicas; in symbiose *Orthezia insignis* Douglas. In foliis *Cassia nodosa. Anacardium occidentale L.* et al.

Trichomerium hirtellum n. sp.

Capnodiaceas

Micelio epifilo, não setoso, membranoso, negro com hifas fuscas, constituidas por celulas de 7,5—12,5 × 3,75—5 u, sem hifopodios, causando revestimento completo da epiderme foliar.

Peritecios setosos, globosos ou piriformes, 90—150 u, de consistencia carnosa e estrutura parenquimatico-membranosa; paredes periteciais formadas por celulas arredondadas, de 5,2—11,7 u; ocorrem esparsa ou, gregariamente; setas do peritecio continuas ou uma ou outra septada, de 56—65 × 5,2—5,6 u; celulas das setas até com 14 u de extensão. Fig. 12 (Pl. XXX).

Ascos cilindricos ou cilindro-clavados, curto pedicelados, octosporos, numerosos e em agrupamento corimboide, 56—78,4 × 8,4—14 u; pedicelo de 8,4 u de comprimento; parafises ausentes. Fig. 13 (Pl. XXX).

Ascosporos disticos a polisticos, hialofragmos, fusoides, com dois septos; polos agudos, 14,4—19,6 × 5,8—8,4 u.

Esporos triposporium presentes.

Picnidios de paredes prosenquimaticas, lembrando a fórma de garrafa, com a altura de 182—351 u; o bojo tem o diametro de 20—37,5 u e o pescoço de 7,5—12,5 u.

Picnidiosporos bacilares ou globosos, hialinos, aglutinados em bola, em tôrno do ostiolo, 3,46—6,92 × 1,73—3,46 u.

Ocasionando fumagina em folhas de *Persea gratissima* Gaert abacateiro.

Material tipo no 1162 e prep. microscopicas de no 760, 761, 762, 763, 764, 765, 766, 767, 768.

Mycelium epiphyllum, pelliculosum, nigrum, ex cellula 7,5—12,5 × 3,75 — 5 u.

Perithecia setosa, globosa, nigra, carnosa vel membranosa, 90—150 u diam; parietibus ex cellulis globosis, 5,2—11—7 u; setis perithecialis simplicis, continuis vel septatis, 56—65 × 5,2—5,6 u.

Ascis cylindro-clavatis, octosporis, 56—78,4 × 8,4—14 u — curto pedicellatis, 8,4 u, non paraphysatis.

Ascosporis distichis vel polistichis, fusiformibus, pluriseptatis, hyalinis, 14,4—19,6 × 5,8—8,4 u.

Spermogonia sessilia, nigra, numerosa, collo longo, 20—37,5 × 182—351 u. Sporidia hyalina, bacillar vel globosa, in substantia gelatinosa agglutinata, 3,46—6,92 × 1,73—3,46 u.

In foliis vivis *Persea gratissima* Gaert.

Typus 1162 in Secção de Fitopatologia—Instituto de Pesquisas Agronomicas—Brasil.

Capnodium hirtum Speg.

Capnodiaceas

Micelio superficial bem desenvolvido, epifilo, formado por hifas septadas, dematioides, fuligineas, sem hifopodios.

Peritecios negros, globosos, estromaticos, sesseis, sobre subiculo, ornados com cerdas que medem 79 × 5,6 u; esparsos, raramente gregarios, de 115 u de diametro. Estrutura pseudo-parenquimatica, com celulas arredondadas.

Ascos sulglobosos, octosporos, polisticos, aparafisados, 45 × 15 u.

Ascosporos fragmosporos, clavados, com tres septos, constritos, 15 × 16 u.

Picnidios, longamente, estiptados, negros, de estrutura prosenquimatica, com ostiolo fimbriado, expulsando espermatias diminutas; medem 130—140 × 30—40 u, variando o colo de 300—325 × 20 u.

Isolado sôbre folhas de louro pardo-*Cordia exselsa* — Jardim Zoo-Botanico. Dois Irmãos. Recife. Pern.

Mat. herb. no 1212.

Chaetopotius, n. gen.

Capnodiaceas

Subiculo negro, efuso, de hifas dematioides, epifilo, formando leve membrana que recobre por inteiro a epiderme foliar; as hifas — são fuscas e repentes; setas presentes no subiculo, embora escassas, não diferentes das hifas do micelio.

Peritecios negros, diminutos, globosos, sesseis e setosos, de situação esparsa sobre o subiculo; paredes periteciais subparenquimaticas, de celulas arredondadas, um tanto transparentes; ostiolo distinto, porém mascarado por perifises.

Ascos de forma clavada, aparafisados, mais ou menos evanescentes, sesseis; a parte basal é estreitada, enquanto o apice é rotundo; contém oito esporos.

Ascosporos cilindro-fusoides dispostos paralelamente em duas fileiras, nos ascos; hialofragmos, com dois septos, sendo então triloculares.

Picnidios de paredes laxas, formadas por hifas que quasi conservam a sua individualidade, conforme se nota ao esmagamento entre lamina e laminula; cilindricos, alongados, negros, com a extremidade livre não fimbriada.

Conidios pequenos, hialinos, cilindrico-fusoides, expelidos em cirro, uniseptados.

Esporos triposporium presentes.

Este genero é criado para conter a especie *C. commistum*, cujas caracteristicas se afastam dos outros generos dos Chae tothyrias, pelo subiculo negro abundante e ocurrencia do estágio picnidico.

A sua posição é intermediaria entre *Antenella* e Chae tothyrium.

Chae topotius n. gen. Subiculum nigrum, setulosum, effusum, pelliculosum. Peritheciis minutissimis, pilosis, nigris, globosis, non appendiculatis, sessilis, ostiolo praeditis. Ascis clavatis, octosporis. Ascosporis cilindrico-fusiformibus, trilocularibus, hyalinis. Spermogonia sessilia, nigra, cylindrica, longa.

Conidiis bicellularis, hyalinis, in substantia gelatinosa agglutinata.

Chaetopotius commistum n. sp.

Capnodiaceas

Subiculo negro, efuso, consideravelmente desenvolvido, epifilo, ocupando toda a superficie foliar, á maneira de delicada membrana.

Hifas dematioides, constritas, repentes, de pequena extensão, fuscas, septadas, de ramificação irregular, constituidas por celulas, de tamanho variavel entre 5—15 × 3,75—5 u. Fig. 19 (Pl. XXXII). Setas do micelio ocorrendo em torno dos peritecios, em pequena quantidade.

São cilindricas, de membrana estreita, não ramificadas e de apice obtuso, não diferençadas das hifas do micelio pte dito; chegam — até a 366,3 × 5—7,5 u e as suas celulas são bastante longas, de 15—22,5 u.

Peritecios negros, globosos ou esfericos, diminutos, medindo de 45—57,5 u de diametro. São sesseis e superficiais, isolados ou gregarios, nunca de disposição cespitosa; ostiolo distinto, ainda que dificilmente visivel, pela proteção que lhe emprestam as perifises. Paredes periteciais subparenquimaticas, um tanto transparentes e de — consistencia membranosa, constituidas por celulas arredondadas, de 3,75—75 u de diametro. Fig. 14 (Pl. XXX).

Apresentam-se ornados de setas cilindricas, um tanto rijas, de 33—46,2 × 3,3—5 u com celulas de 7,5—10 u de comprimento, por 3,75—5 u.

Ascos clavados, de apice rotundo e base conica, octosporos, mais ou menos evanescentes e pouco numerosos, de 32—42 × 10—19,6 u; sesseis. Fig. 15.

Ascosporos cilindro-fusoides, situados, paralelamente, em duas fileiras, nos ascos; hialofragmos, com dois septos, tricelulares portanto; polos agudos, 15—18,75 × 4,3—5 u.

Picnidios abundantes, de paredes laxas, resultantes de uma parcial fusão entre as hifas do subiculo que se especializaram na construção dessa estrutura; ao esmagamento entre lamina e laminula pode ser observada a independencia dessas hifas. São cilindricos, de — 399—456 × 14,8—19,6 u., negros, com o bico não fimbriado. Figs. 16, 17, 18 (Pl. XXXI).

Conidios hialinos, de pequeno tamanho, cilindrico-fusoides, hialodidimos, de 8—4 u. de diametro, libertados em cirro.

Esporos triposporium presentes.

Essa especie ha sido encontrada sobre caiuia-*Clidemia hirta* (*L*) D. Don da familia das Melastomaceas, de mistura com *Netrocymbe depressum.*

Consideramo-la como tipo, sendo a localidade tipo o bairro de — Monteiro, no Recife.

Acha-se herbarizado o material, na Secção de Fitopatologia, do I.P.A., sob nos 1159 e 1161 e p. u. 749, 750, 751, 752, 753, 754 e 755.

Subiculum epiphyllum, nigrum, effusum. Hyphae fuscae, septatae, repentae, irregulare-ramificatae, cellulis 5—15 × 3,75—5 u. Setae hypales, rectae, simplices, septatae 366,3 × 5—7,5 u; cellulis apicalibus subulatis- Peritheciis minutissimis, nigris, globosis ,— 45—57,5 u in diam, sessilis, membranaceis, parietis ex cellulis globosis, 3,75—7,5 u; ostiolo praeditis.

Setis perithecialis simplices, 33—46,2 × 3,3—5 u. Ascis clavatis, ad apicem rotundatis, octosporis, 33—42 × 10—19,6 apedicellatis. Ascosporis fusiformibus, trilocularibus, hyalinis, 15—18,75 × 4,3—5 u.

Spermogonia cylindrica, nigra, 229—456 × 14,8—19,6 u.

Conidiis hyalinis, bicellularis, fusoidis, 8—4 u, in substantia gelatinosa agglutinatis.

Ad folia viva *Clidenia hirta* (L) D. Don; associata *Netrocymbe* de pressum n. sp; Typus 1159 in Secção de Fitopatologia, Instituto de Pesquisas Agronômicas, Brasil.

Setella coracina n. sp.

Capnodiaceas

Subiculo negro, efuso, com hifas dematioides, de côr marron, formadas por celulas de 11,2—16,8 × 4,2—5,6 u; as hifas são reunidas, algumas vezes, em compactos feixes, ordinariamente lisos, ou levantadas, setiformes, com 280 × 8,4 u; situação epifila. Hifopodios ausentes.

Corpos de frutificação estromaticos, e de dois tipos: ascigero e espermogonico, assentes sôbre o subiculo.

Os estromas ascigeros são globosos ou piriformes, sesseis, de 86,8—100,8 u, apresentando reflexos metalicos. Esses estromas são uniloculares, de paredes ásperas e negras, carbonaceas-membranosas, providas de setas de tamanho variavel 70—75,6 × 5 u e com celulas de 7—14 u; ausencia de ostiolo definido. As celulas das paredes periteciais têm 4,2—8,4 u de diametro. Fig. 20 (Pl XXXII).

Ascos elipticos, aparafisados, apedicelados, de apice quasi acuminado, com oito esporos, e medindo 39,2—53,2 × 14—16,8.

Ascosporos fracamente coloridos, mas tomando intensa coloração azul quando as preparações são coradas pelo Azul de Aman; septados, com 3—6 septos, elipticos-fusoides, recurvados ou retos, — 16,8—22,4 × 4,2—5,6 u.

Estromas espermogonicos igualmente negros e brilhantes, globoso-cilindricos, colo longo e apice fimbriado, lembrando a frutificação peritecial de *Ceratostomela,* e de estrutura pletenquimatica, variando a altura total de 210—330 u; a parte globosa ou bojo tem de 112,8—126 × 84—89,6 u e o pescôço a altura maxima de 224 u para um diametro variavel entre 16,4—25 u. Fig. 21. (Pl. XXXII).

Conidios fusoides, fuscos, de polos acuminados, multiseptados, 30,8—56 × 5,6—8,4 u. Fig. 22 (Pl. XXXII).

Conidioforos da base dos picnidios fuligineos e ramificados.

Esporos triposporos presentes.

Isolado sobre *Mauritia vinifera,* em associação com Netrocym be *Mauritiae n. sp.*

Mat. tipo 1154 e p. m. 737, 738, 739, 740, 741 e 742.

Tambem isolado sobre *Clitoria racemosa* — 1181; sobre bambú *Bambusa arundinacea* — 1183, em simbiose com *Cerataphis lataniae* Boisduval 1187.

S/ juçara, em simbiose com *Orhtezia insignis* Douglas; s/ jaqueira — *Artocarpus integrifolia L* c/ *Vinsonia stellifera* Westwood e *Pseudaonidia trilobitiformis* Green. mat. 1191 — Dois Irmãos.

S/ *Cyperus* ? 1258 com *Netrocymbe inspersum* n. sp.

S/ *Erythroxylon* sp. — 1278.

S/ alfavaca de cobra — *Monnieria trifolia* L.

Subiculum nigrum, effusum; mycelium ex hyphis dematioide, brunneis, cellulis 11,2—16,8 × 4,2—5,6 u, anastomosantis quase rectis vel repentis; epiphyllo.

Setulas sparsas, fuscas, usque 280 × 8,4 u. Peritheciis globosis vel pyriformibus, sessilibus, 86,8—100,8 u diam, carbonaceo membranaceis, asperatum ad superficiem, pilosulis, 70—75,6 × 5 u, ex cellulis 7—14 u. Ascis ellipticus apicem quase acuminatis 39,2—53,2 × 14—16,8 u, 8 sporis, apedicellatis; paraphysibus nullis.

Ascosporis elliptico-fusoides, 3—6 septatis, 16,8—22,4 × 4,2—5,6 u, coloratis. Spermogonia sessilia, nigra, globosa-cilindraceis, 112,8—126 × 84—89,6 u diam. collo longo apicem fimbriata, 224 × 25 u — 16,4 u.

Sporidia fusiformibus, pluriseptatis, apicem acuminatis, fusca, 30,8—56 × 5,6—8,4 u. Triposporium praesentis.

In foliis vivis *Mauritia vinifera.*

Typus 1154 in herbarium Secção de Fitopatologia, Instituto de Pesquisas Agronomicas.

Chaetomeris hemisphaerica n. sp.

Capnodiaceas

Subiculo tenue membranoso, a principio escasso, depois abundante, formando delicada pelicula, mais ou menos transparente, que se torna fusca depois. Hifas vegetativas dematioides, um tanto constritas, fuscas, com celulas de 14—16,6 × 5,6—8 u; ausencia de hifopodios.

Distribuição epifila. Aqui e ali o subiculo origina pequenas verrugas, parenquimaticas cuja porção superior se deprime, á maneira de coxim, acomodando os corpos frutigenos.

Os peritecios são hemisfericos, ostiolados, de 228—285 u de diametro, carnosos ou de consistencia flacida, formados pela aglutinação de celulas monilioides, de 5,6—11,2 u de diametro; estrutura pseudo parenquimatica; esparsos ou gregarios; coloração marron-fuliginea.

Ascos clavados ou oblongos, octosporos, de apice rotundo e base cuneada, pedicelados, sem parafises verdadeiras, mas com parafisoides. Medem 56—64,4 × 28—36,4 u; o pedicelo tem 28 × 8,4; sua disposição no peritecio é basal em agrupamento umbelado. Fig. 23 (Pl. XXXIII).

Ascosporos polisticos, cilindro-fusoides, com um cilio em ambas as extremidades; dictiosporos, apresentando 6 septos transversais e aproximadamente 2 longitudinais; são hialinos, ou levemente fuscos, 30,8—35 × 8,4—11,2 u.

Picnidios ausentes.

Foi isolado em associação com *Netrocymbe Lafoensiae* n. sp. e *Dimeriellopsis araliae* n. sp. sôbre folhas de "croton tapa muro" — *Polyscias gnifoylei* Bailey.

Mat. herb. 1169 e p. m. 768, 782, 793, 794, 795.

Simbiose com *Orthezia insignis* Douglas, constatada. *Chaetomeris*, corresponde a *Treubiomyces* v. Hohn, mudado por Th e Syd.

Subiculum epiphyllum, effusum, pelliculosum, fuscum, ex hyphis constrictis; ex cellula 14—16,6 × 5,6—8 u.

Perithecia hemisphaerica, 228—285-diam, ostiolata, carnosa vel membranosa, fusca, sparsa vel gregaria; parietibus ex cellulis monilioides, 5—6—11,2 diam.

Ascis clavatis vel oblongis, octosporis, apice obtusis, — 56—64 × 28—36,4 u, pedicellatis 28 × 8,4 u.

Ascosporis fusiformibus, polistichis, hyalinis vel fuscis, muriformibus, cum cilia polar, 30,8—35 × 8,4—11,2 u.

Pycnidiis absentis. u (see l.K)

In foliis vivis *Polyscias gnifoylei* Bailey.

Typus 1169 in Secção de Fitopatologia do Instituto de Pesquisas Agronomicas-Brasil.

ocymbe !

Netrocymbe depressum n. sp.

Capnodiaceas

Subiculo epifilo, esponjoso-crustaceo, negro, de hifas dematioides, cujas celulas se organizam á maneira monilioide; o subiculo é mais ou menos laxo, oferecendo aqui e ali aspecto aracnoide. Individualmente, as hifas miceliais são fuligineas, retas e intercruzadas, 2,8—5,6 u de diametro; as celulas monilioides variam de 11,2—14 de extensão.

O subiculo acha-se, intimamente, ralacionado á vegetação de *Chaetopotius commistum n. sp.* numa associação tão estreita que não parece acidental, no material tipo.

Disposição epifila sobre a matriz.

Peritecios globosos, de comêço, depois, de colapso deprimido, sesseis, negros, não setosos, ocorrendo de modo isolado; paredes periteciais de estrutura pletenquimatica, apresentando-se de consistencia membranosa, flacida; esmagando-se, verifica-se que essas paredes são formadas pelo ajuntamento de hifas do subiculo; celulas parietais globosas, de 7,5 u de diametro.

Maduros, os peritecios medem de 168—380 u de diametro sem haverem tornado distinto o seu ostiolo, que se acha localizado sôbre pequena papila. Fig. 24 (Pl. XXXIII).

Ascos cuneiformes, pedicelados, situados umbeladamente nos peritecios; medem de 64,4—75,6 × 14—28 u; pedicelo de 14 u de extensão; são octosporos. Parafises presentes, filiformes. Ascosporos, de inicio, fusoides e hialinos, mais tarde fuscos; muriformes, com x septos transversais; disposição polistica, 33,6—53,2 × 14—16,8 u. Fig. 25 (Pl. XXXIII).

Isolado, juntamente com Chae topotius *commistum* n. sp., sobre folha de caiuia-*Clidemia hirta* (L) D. Don.

Mat. herb. no 1159 e p. m. 743, 744, 745, 746, 747 e 748.

Subiculum epiphyllum, nigrum, quase crustaceum, arachnoideum, densum. Hyhis septatis, leviter ad septa constrictis, fuscis, rectis, 2,8—5,6 u diam, ex cellula moniliodis, 11,2—14 u.

Peritheciis globosis, papillatis, 168—380 u diam, membranaceis, parietibus ostiolatibus et collabentibus. Ascis cuneiformis, 64,4—75,6 × 14—28, octosporis, pedicellatis; paraphysibus filiformibus. Ascosporis polystichis, fusiformibus, hyalinis late fuscis, muriformibus, X septa transversalibus, 33,6—53,2 × 14—16,8 u.

In foliis vivis *Clidemia hirta* (L.) D. Don., associatis Chae topotius *commistum* n. sp. Typus 1159 in Secção de Fitopatologia, Instituto de Pesquisas Agronomicas. Brasil.

Netrocymbe Mauritiæ n. sp.

Capnodiaceas

Subículo epifilo, negro, efuso, aspero, de hifas constritas, escassamente ramificadas e intercrusadas, mal definidas, em geral, com aspecto crustoso, formando pelicula. Quando em associação com o micelio de *Setella coracina* n. sp., observa-se uma perfeita integração dos sistemas vegetativos desses dois fungos, recobrindo, uniformemente, os orgõas foliares dos suscetiveis.

Peritecios negros, Fig. 1, globosos a principio, papilados, depois, de colapso deprimido, de 400—500 u de diametro, dotados de ostiolo diminuto e esparsos; estrutura pletenquimatica; paredes constituidas pelo aglutinamento de hifas miceliais, podendo-se notar, contudo, algumas celulas individualisadas, com 16,8 × 8,4 u, mais ou menos elipticas; conssistencia branda. Fig. 26 (Pl. XXXIII).

Triposporios presentes. Picnidios ausentes, no material tipo.

Ascos clavados, de apice rotundo, em agrupamento corimboide, medindo de 72,8—84 × 36,4—42 u; octosporos. Parafises presentes, clavuladas. Fig. 27.

Ascosporos muriformes, hialinos de comêço, depois fuscos; em regra, com oito septos transversais e 3—5 longitudinais, oblongo-fusoides, polisticos: 39,2—50,4 × 16,8—19,6 u.

Habitat: S/ *Mauritia vinifera*, produzindo fumagina.

Material tipo no 1154 e preparações microscopicas de nos 730, 731, 732 e 736.

Outras peças no herbario: 1157, s/ *M. vinifera;* 1270, s/ *Vismia sp.* (lacre).

Subículo epiphyllum, nigrum effusum, asperatum ad superficiem, pelliculosum, ex hyphis crustaceus, ramosis, articulatisque torulosis.

Speramogonia absentis. Perithecia goblosa papillata, molliuscula, 400—500 u. dein discoideo-collabentia, sparsa; ostiolum minuta; parietibus pletenchymatous, celula ex hyphis 16,8 × 8,4 u. Ascis clavatis, apice rotundatis, 72,8—84 × 36,4—42 u. octosporis; paraphysibus filiformibus, clavatis. Ascosporis pluriseptato-muriformia, fusoideo-oblonga, polystichis, brunneis ad maturitatem, 39,2—50,4 × 16,8—19,6 u.

In foliis vivis *Mauritia vinifera;* typus no 1154-herbarium Instituto de Pesquisas Agronomicas — Pernambuco — Brasil.

Netrocymbe lafoensiae n. sp.

Capnodiaceas

Micelio negro, efuso, revestindo a superficie foliar por completo, á maneira de uma pelicula, ou crostra, sem hifopodios.

Hifas vegetativas fuligineas, septadas, constritas, algumas vezes monilioides, repentes, raramente setuladas; caracter dematioide bem definido; espessura das hifas variavel entre 2,5—7,5 u; celulas hifais de 5—15 u de extensão. Disposição epifila.

Peritecios esfericos ou globosos, sesseis, negros, gregarios, não setosos, astomos, 140—252 u, de paredes membranosas; celulas das paredes de 3,78—11,34 u. de diametro arredondadas; estrutura parenquimatica.

Ascos a principio cilindro-clavados, depois, oblongos, apedicelados, aparafisados, situados, paralelamente, uns aos outros, quadrisporos, 42—56 × 28—33 u. Fig. 28 (Pl. XXXIV).

Ascosporos eliptico-alongados, feodictos, com 5 septos transversais e x septos longitudinais, fuscos, polisticos, 30,24—44,8 × 12,5—17,5 u.

Picnidios sesseis, numerosos, bojudo-alongados ou simplesmente cilindricos, com as dimensões totais de 148,4—316,4 × 8,4—19,6; o bojo tem, aproximadamente 168 × 19,6 u. As paredes dos picnidios são provenientes da aglutinação de hifas do subiculo, que se tornam bem unidas. Ostiolo não fibrilado. Picnidiosporos bacilares, hialinos, expulsos em cirro mas não agrupados em bola, 2,5—3,42 × 1,73 u. Esporos triposporium presentes. Fig. 29. Isolado sobre folhas de merindiba-*Lafoensia glyptocarpa* Koehne em simbiose com *Orthezia insignis* Douglas.

Mat. herb. 116 e 1167 e p. m. 786, 787, 789, 790 e 791.

Localidade tipo: Dois Irmãos. Recife. Pernambuco.

Tambem isolado em associação com *Trichomerium psidii*, 1888, *Setella coracina*, 1192, sobre erva de rato-*Hamelia patens* Jacq.

Em associação c/ *Chaetomeris hemisphoerica* e *Dimeriellopsis araliae* n. sp.

Sobre planta indet. , no 1251, foi isolado em associação com *Orthezia*, apresentando peritecios abundantes e picnidios maiores, do que os do tipo, com 368—520 u.

S/ *Vismia sp.* c/ *Orthezia insignis* Douglas.

Mycelium nigrum, pelliculosum, epiphyllum, ex hyphis articulatisque constrictis, repentibus, raro setulatis; cellula 2,5—7,5 × 5—15 u.

Perithecia sessilia, nigra, gregaria globosa astoma, non setulata, membranacea, 140—252 u; parietibus ex cellulis globosis, 3,78—11,34 u.

Ascis oblongo-clavulatis, apedicellatis, 4 sporis, 42—56 × 28—33 u. paraphysibus nullis. Ascosporis elliptico-elongatis, coloratis, muriformibus, laevibus, polistichis, 30,24—44,8 × 12,5—17,5 u.

Spermogonia sessilia, numerosa, nigra, collo longo ad apicem non fimbriata, 148,4—316,4 × 8,4—19,6 u. Sporidia hyalina, bacillar, non agglutinata in substantia gelatinosa, 2,5—3,42 × 1,73 u.

In foliis vivis *Lafoensia glyptocarpa* Koehne typus 1166 in herbarium Secção de Fitopatologia-Instituto de Pesquisas Agronômicas.

Netrocymbe perparum n. sp.

Capnodiaceas

Micelio negro, escasso, epifilo, de hifas fuscas, bastante constritas e intercruzadas, tendo celulas de 10—17,5 × 5—7,5 u; o aspecto de conjunto é aparentemente esponjoso.

Peritecios negros, astomos, globosos, mas de colapso deprimido á maturidade, glabros, de estrutura pseudo-parenquimatica e consistencia membranosa. Fig. 30 (Pl. XXXIV).

Medem de 125—210 u tendo as celulas periteciais de 5—10 u.

Ascos clavados apedicelados, aparafisados, quadrisporos ou octosporos, 52,5—67,5 × 27,5—32,5 u. Fig. 31 (Pl. XXXV).

Ascosporos elipticos, hialinos na juventude, depois fuscos, muriformes, com 7 septos transversais, de 35—42,5 × 10—12,5 u.

Picnidios ausentes.

Isolado sôbre alfavaca de cobra, ou de caboclo-*Monnieria trifolia* L. em associação com *Orthezia insignis* Douglas.

Mat. herb. no 1257 e 1349.

S/ *Bougainvillea spectabilis.*

Mycelium nigrum, epiphyllum; hyphae fuscae, constrictae ramificatae, ex cellulae 10—17,5 × 5—7,5 u.

Perithecia nigra, astoma, membranosa, globosa, glabra, — 125—210 u; collabentibus ad maturitatem; parietibus ex cellulis globosis, 5—10 u diam.

Ascis clavatis, apedicellatis, aparaphysatis, quadrisporis vel octosporis, 52,5—67,5 × 27,5—32,5 u.

Ascosporis muriformibus, colaratis, pluri septatis (7 transv) 35—42,5 × 10—12,5 u.

Picnidiis absentis.

In foliis vivis *Monnieria trifolia* L. cum *Orthezia insignis* Douglas. Typus 1257, in Secção de Fitopatologia-Instituto de Pesquisas Agronômicas-Brasil.

Netrocymbe inspersum n. sp.

Capnodiaceas

Micelio efuso, negro, membranoso, de hifas monilioides tipicas, fuscas, com celulas de 8,4—16,8 × 5,6—8,4 u; hifopodios ausentes; distribuição epifila, revestindo parcial ou totalmente á página superior das folhas.

Peritecios esfericos, negros, astomos, assentes sôbre frouxa trama hifal, esparsa, não setosos, de estrutura pseudo parenquimatica e consistencia membranosa, pequenos, 140—168 u. Fig. 32 (Pl. XXXV).

Paredes periteciais formadas pelo aglutinamento de hifas do micelio, e integradas por celulas arredondadas, de 8,4—14,0 u de diametro.

Ascos elipticos, sesseis, aparafisados, em agrupamento umbeliforme, 56—70 × 30,8—36,4.

Ascosporos feodictos, polisticos, oito por asco, 44,8—56 × 11,2—14 u. manifestando-se sub-hialinos a principio e depois tornando-se olivaceos.

Picnidios cilindricos de paredes laxas e bico não fimbriado 154—280 × 14—22 4 u abundantes.

Picnidiosporos globosos expelidos mais ou menos livremente, hialinos e diminutos, escassos 1,73—3 46 u.

Triposporium presente.

Associado a *Trichomerium* sp. do qual só uma frutificação foi encontrada.

A especie tipo está representada pelo material de no 1252 e p.m. 943, 944.
Localidade tipo: Caxangá.
S/ 1258 c/ *Setella coracina n. sp.*
S/ Imbira vermelha-(*Xylopia frutescens*)—1279—Casa Amarela.
S/ *Bougainvillea glabra*, c/ *Orthezia insignis* 1280
S/ Avenca de cacimba-*Adianthum sp.*
S/ meladinha — indet.

Mycelium effusum, nigrum, ex hyphis articulatisque torulosis, constrictis; cellula 8,4—16,8 × 5,6—8,4 u. Perithecia globosa nigra, astoma, sparsa, membranosa, pseudo-parenchymatica 140—160 u diam; parietibus ex cellulis globosis 8,4—14 u diam.

Ascis ellipticis apedicellatis aparaphysatis 56—70 × 30,8—36,4 u. Ascosporis muriformibus, fuscis, octosporis, polystichis 44,8—56 × 11,2—14 u. Spermogonia cylindrica, sessilia, nigra, ad apicem non fimbriata, numerosa, 154—280 × 14—22,4 u.

Sporidia globosa, hyalina, diminuta, 1,73—3,46 u.

Ad folia viva *meladinha*, pl. indet. et. *Xylopia frutescens, Bougainvillea glabra, Adianthum sp.*

Typus no 1252 in Secção de Fitopatologia-Instituto de Pesquisas Agronomicas-Pernambuco-Brasil.

Netrocymbe robusta n. sp.

Capnodiaceas

Subiculo espesso, negro, de ocurrencia epifila, revestindo a epiderme da pagina superior das folhas bem como o peciolo e ramos. Hifas vegetativas constritas, fuscas, ramificadas e intercruzadas, sem hifopodios, possuindo celulas de 12,5—20 × 6,25—8,75 u; setas ausentes.

Peritecios raros sôbre as folhas, mas abundantes sobre os ramos; globosos, negros, de colapso deprimido, glabros, sem ostiolo definido, gregarios ou até cespitosos, 266—361 × 266—280 u. Fig. 33 (Pl. XXXV).

Paredes periteciais constituidas por celulas arredondadas, de 9,8—14 u, apresentando estrutura parenquimatica; consistencia membranosa.

Ascos primeiro cilindraceos, depois clavados, com 4 ou 8 esporos, pedicelados, parafisados, de agrupamento basal-umbelado, 75,6—98 × 36,4—43,6 u; pedicelo até 56 u de extensão; parafises filiformes. Fig. 34 (Pl. XXXV).

Ascosporos muriformes, fuscos-olivaceos, disticos, oblongo-fusoides, com 3—5 septos transversais, 44,8—56 × 16,8—22,4 u.

Picnidios em garrafa, alongados, 208—338 × 20—30 u, no bojo; o pescoço tem de 100—125 × 7,5—10 u; paredes prosenquimaticas; bico fibrilado; picnidiosporos elipticos, hialinos, 2,5 × 1,25, libertados em cirro e agrupados em bola.

Esporos triposporium presentes.

Em associação simbiontica com *Orthezia insignis* Douglas. Isolado sobre *Solanum robustum* Wendel-jurubeba.

Tipo integrado pelo material de no 1185 da Secção de Fitopatologia do I.P.A. Localidade tipo: Dois Irmãos. Recife-Pernambuco. p. m. 818 a 823.

Subiculum crassum, nigrum, epiphyllum; mycelium ex hyphis constrictis, fuscis, ramificatis, cellulis 12,5—20 × 6,25—8,75 u.

Peritheciis in foliis et in ramis, nigris, atris, globosis, membranaceis, ex hyphis parietibus globosis, 9,8—14 u, collabentibus ad maturitatem, 266—361 × 266—280 u. non ostiolatis.

Ascis cylindro-clavatis, quadri vel octosporis, paraphysatis, 75,6—98 × 36,4—43,6 u, longo pedicellatis, 56 u.

Ascosporis muriformibus, fuscis, oblongo-fusoidis, distichis, pluri-septatis (3—5 transv.) 44,8—56 × 16,8—22,4 u.

Spermogonia atra, nigra, sessilia, numerosa, collo longo ad apicem fimbriata, 208—338 × 20—30 u; sporidia hyalina, continua, ellyptica, in substantia gelatinosa, 2,5 × 1,25 u.

In ramis et in foliis vivis *Solanum robusta* Wendel cum *Orthezia insignis* Douglas.

Typus 1185 in Secção de Fitopatologia, Instituto de Pesquisas Agronômicas-Brasil.

Phaeopeltis sapotae n. sp.

Capnodiaceas

Micelio, negro, abundante, crustaceo membranoso, revestindo por completo a pagina superior das folhas de *Achras sapota* L.

Suas hifas são fuscas, septadas, com celulas, de 15,12—22,68 × 3,78—5,67 u, de quando em quando ramificadas crucialmente; em geral, são mais direitas do que dematioides. Hifopodios e setas ausentes.

Conidios fragmosporos (triposporio) pluriloculares, constritos, de apice atenuado e base rotunda, 75,60 × 7,56 u.

Peritecios a principio globosos, depois de colapso deprimido, apresentando, porém, ostiolo bem definido; coloração negra; estrutura pseudo-parenquimatica celulas parietais mais ou menos arredondadas, de 3,78—7,56 u de diametro são glabros e relativamente escassos sobre o micelio; suas dimensões se mostram dentro nos limites de 170—189 u de diametro.

Ascos ovalados, aparafisados, de 34—45,36 × 18,90—34 u, octosporos. Ascosporos elipticos, de polos bem rotundos, direitos ou obliquos, hialodictos e polisticos, 22—30 × 7—11 u.

Picnidios cilindraceos, direitos, algumas vezes ramificados, tendo o apice indistintamente fibrilado; estrutura prosenquimatica; dimensões de 189—291 × 18—19 u.

Picnidiosporos bacilares, expulsos em cirro, 3,22 × 1,61 u.

Mat. colhido sôbre *Achras sapota* L. em associação simbiontica com *Pseudaonidia trilobitiformis* Gréen, pelo Agro. José Nery, em Aflitos — Recife.

Mat herb. na Secção de Fitopatologia do I.P.A., sob os nos 226 e 1353.

Mycelium nigrum, crustaceum vel membranaceum, ex hyphis fuscis, septatis, perpendiculariter vel cruciater ramificatis; cellula 15,12—22,68 × 3,78—5,67 u.

Peritheciis globosis collabentis ad maturitatem, nigris, 170—189 u diam; parietibus ex cellulis globosis, 3,78—7,56 u, ostiolo nitidulo. Ascis ovoideis, aparaphysatis, octosporis, 34—45,36 × 18,90—34 u.

Ascosporis muriformis, hyalinis, 22—30 × 7—11 u.

Spermogonia cylindrica, simplicis vel ramificatis, 189—291 × 18—19 u. Picnidiosporis bacillaris, hyalinis, in substantia gelatinosa agglutinata, 3,22 × 1,61 u.

Triposporium plurilocularibus, constrictis 75,60 × 7,56 u.

Ad folia viva *Achras sapota* L. cum *Pseudaonidia trilobitiformis* Green-Typus 1353, in Secção de Fitopatologia-Instituto de Pesquisas Agronômicas. Pernambuco-Brasil.

Limacinia capsici n. sp.

Capnodiaceas

Micelio escasso, tenue, membranoso, recobrindo parcial ou totalmente a pagina superior das folhas. Hifas vegetativas dematioides, torulosas, ramificadas, com celulas de 7,5—17,5 × 3,75—7,5 u. ausencia de setas e de hifopodios.

Peritecios globosos, 226—372,4 u, negros, assentes sobre pequeno subiculo á maneira de coxim; sesseis, glabros, esparsos ou gregarios. Paredes parenquimaticas-membranosas, com celulas arredondadas, formando uma camada, aparentemente, de 5—10 u de diametro; ostiolo indefinido; coloração negra.

Ascos numerosos, em disposição basal umbelada, 35—50 × 10—12,5 u, aparafisados, apedicelados, cilindro-clavados, octosporos.

Ascosporos hialofragmos, cilindro-fusoides, de polos agudos, polisticos, de 15—20 × 3,75—5 u.

Picnidios em garrafa, alongados, ocorrendo tambem com bojo mediano ou terminal; paredes de estrutura prosenquimatica; bico fibrilado. Altura total 247—308 u; largura do bojo de 25—45 u e do pescoço de 12,5—16,8 u.

Picnidiosporos hialinos, bacilares, 2,5—5 × 1,25 u.

Esporos triposporium ausentes.

Isolado sôbre pimenta malagueta-*Capsicum frutescens* L., em vida simbiotica com *Orthezia insignis* Douglas.

O tipo está representado pelo material de n 1182, da Secção de Fitopatologia, do I.P.A. p. m. 813, 814, 815, 816, 817.

Localidade tipo; Dois Irmãos. Recife. Pernambuco.

Mycelium nigrum, membranaceum, epiphyllum, ex hyhis, articulatisque torulosis, cellulis 7,5—17,5 × 3,75—7,5 u.

Perithecia globosa, 226—372,4 u, sessilia, glabra, sparsa vel gregaria, nigra; parietibus ex cellulis globosis, 5—10 u.

Ascis numerosis, aparaphysatis, apedicellatis, cylindro clavatis octosporis, 35—50 × 10—12,5 u. Ascosporis cylindro-fusoidis, pluriseptatis, hyalinis, 15—20 × 3,75—5 u.

Spermogonia sessilia, nigra, collo longo, ad apicem fimbriata, 247—308 × 25—45 u; sporidia hyalina, continua, 2,5—5 × 1,25 u.

In foliis vivis *Capsicum frutescens* cum *Orthesia insignis* Douglas.

Typus 1182 in Secção de Fitopatologia-Instituto de Pesquisas Agronomicas — Pernambuco. Brasil.

Limacinia aurantii P. Henn.

Capnodiaceas

Micelio efuso, crustaceo-membranoso, apresentando hifas dematioides, de dois tipos: um claro, de longa extensão, não ramificado, de aspecto aracnoide quando visto á lupa, com céludas de — 12,5—25 × 7,5—10 u; o outro, fuligineo, densamente intercruzado e é o que origina, propriamente a caracteristica do micelio; epifilo e sem simbiose com insetos; desprovido de setas.

Peritecios subhemisfericos ou globoso-deprimidos, membranosos ou subcoriaceos, emergentes a principio, depois superficiais, sesseis; glabros e astomos, medindo o seu diametro de 180—200 u; estrutura parenquimatica; celulas parietais arredondadas, de 10—15 u de diametro; fuscos. Fig. 35 (Pl. XXXVI).

Ascos cilindraceos para clavados, sesseis, de apice rotundo e tunicados, numerosos, aparafisados, octosporos e polisticos, 50—60 × 20—25 u. Fig. 36.

Ascosporos hialofragmos, triseptados, fusoides, com um polo obtuso e outro agudo, conglobados, ou seja dispostos ao modo de novêlo nos ascos, 20—30 × 5—7,5 u.

Esporos triposporium ausentes.

Igualmente, ausente o estagio imperfeito do fungo.

Isolado sôbre folhas de aticum-*Anona montana* Macf., Casa Amarela-Recife.

Mat. herb. no 1271 e p. m. 972, 973, 974, 975, 978, 979.

S/ Sambaquim-*Didymopanax morototonis* Mat no 1274.

Meliola bicornis (Wint) var. lonchocarpii n.v.

Meliolaceas

Micelio epifilo, tenue, sepia, constituindo manchas circulares, até de 8 mm de diametro, as quais, frequentemente, se tornam — confluentes Fig. 37; hifas retas ou ramificadas, com aspecto arborescente, fuscas, de 7,5—10 u de espessura septados; hifopodios alternos ou opostos, ovoides ou elipticos, obtusos, 13,75—17,5 × 10—15 u, com celula basal cilindrica 7,5 × 7,5—10 u. Setas eretas, abundantes, erguendo-se do micelio, fuligineas, de septos não diferençados, 180—220 × 3—4 u, dicotomicas ou esgalhadas. Em geral, o ramo primario tem 142,5 u e os secundarios de 60—80 u; a porção terminal das ramificações é clavulada, tendo a celula de 20—37,5 × 10—15 u.

Cleistotecios globosos, negros, de estrutura pseudo-parenquimatica, com celulas parietais fuscas, de 7,5—17,5 u, opacas, mostrando-se com leve colapso, quando maduros e de superficie rugosa; diametro de 110—167 u.

Setas cleistoteciais não distintas das do micelio. Fig. 38 (Pl. XXXVII).

Ascos elipticos, com dois esporos, situados paralelamente, mas, um a dois terços do comprimento do outro; apedicelados; de paredes evanescentes; 47,5—52 × 30—32,5 u; aparafisados.

Ascosporos cilindrico-oblongos, fuscos, com 5 celulas, constritos nos septos, lisos e de polos rotundos, retos e curvos, 47,5—50 × 17,5—20 u. Fig. 39.

Esse fungo difere da especie *M. bicornis*, na tipologia das setas e no tamanho dos ascosporos, afastando-se tambem, completamente, de todos os outros Meliola. Como os demais caracteres concordam com os da especie mencionada, que já foi identificada sobre *Lonchocarpus glaucifolius* Urb. e o fungo ora descrito foi encontrado sobre *Lonchocarpus sp.* preferimos torná-lo variedade, em vez de criar uma especie nova. O material que serviu ao nosso estudo está classificado na Secção de Fitopatologia do I.P.A. sob no 504 e p.m.

Mycelium epiphyllum, saepia, in plagulas circularibus, 1—8 mm diam, sparsa vel confluentibus; ex hyphis rectis vel arborescentis, fuscis, 7,5—10 u diam. Hyphopodia alterna vel opposta, ovoide vel elliptica, 13,75—17,5 × 10—15 u, cellula basale 7,5 × 7,5—10 u.

Setae hyphales 180—220 × 3,4 u longae septatae, ad apicem ramificatis. Setis cleistothecialis conformibus. Cleistotheciis globosis, astomis, 110—167 u, collabentibus, parietibus ex cellulis polygonalis, 7,5—17,5 u. diam.

Ascis ellipticis, bisporis, apedicellatis, aparaphysatis, 47,5—52 × 30—32,5 u.

Ascosporis cylindrico-oblongis, constrictis, fuscis, 5- septatis, rectis vel recurvis, 47,5—50 × 17,5—20 u.

Ad folia viva *Lonchocarpus* sp.

Typus 504 in Secção de Fitopatologia-Instituto de Pesquisas Agronômicas-Pernambuco-Brasil.

Meliola anacardii Zimm.

Meliolaceas

Micelio reduzido, dendritico, originando manchas esparsas, arredondadas de 2,5 a 3,8 mms, epifilo, com hifas organizadas por celulas de 12,5—20 ×

5—7,5 u. Essas hifas são fuligineas e de ramificacão oposta. Fig. 40. Setas do micelio septadas, 175—190 × 7,5 u.

Hifopodios presentes, elipticos ou verruciformes com a celula basal mais curta do que os articulos; medem de 10—16,25 × 7,5—10 u enquanto a celula referida varia de 3,75—6,25 × 2—6,25 u. Fig. 41 (Pl. XXXVII).

Assentes sobre o micelio, acham-se os peritecios que podem ser vistos com facilidade mesmo sem o auxilio de lupa. Os peritecios são subglobosos, achatados, 90—150 u, membranosos, setosos, de paredes parenquimaticas; as celulas dessas paredes têm de 7,5—15 u de diametro; as setas medem 156—195 × 7,5 u.

Os ascos são apedicelados, aparafisados, elipsoides, evanescentes, contendo apenas dois esporos, 40—50 × 25—27,5 u. Fig. 42 (Pl. XXXVIII).

Os ascosporos são cilindroides, ou elipticos, um tanto curvos, de polos rotundos, hialinos a principio, depois fuligineos, quadriseptados e constritos nos septos, 35—42,5 × 12,5—17,5 u.

Os caracteres desta especie aproximam-se dos do fungo por nós colhido, razão pela qual preferimos considerá-lo como um representante da mesma.

Isolado sobre folhas de *Anacardium occidentale* L — cajueiro.

Mat. herb. no 1163 e p. m. 769, 770, 771, 772, 773.

Meliola bidentata Cooke

Meliolaceas

Micelio escasso, dendritico, formando manchas espalhadas, mais ou menos circulares, de 1—4 mms de diametro, as quais, frequentemente, se tornam confluentes; disposição sempre epifila.

Hifas vegetativas fuligineas, intercruzadas, com celulas de 16—25,76 × 5,64—6,44 u. Setas do micelio numerosas, direitas, com o apice bidentado, ainda que algumas vezes possa se mostrar como tridentado, com a ocurrencia de mais um dentículo na base das saliencias tipicas; a septação das setas é bem nitida, individualizando celulas de 49—53 × 7,56—9,45 u.

A altura total dessas estruturas é de 340—359 × 7,56—9,45 u.

Hifopodios presentes, verruciformes, alternos, bicelulares, com a celula basal de menor tamanho: 3,78 × 5—6; a celula apical tem de 7,56—11,34 × 5—6 u.

Peritecios, regularmente, sôbre o micelio, globosos ou achatados, 132—189 u de diametro, setosos, de estrututa parietal parenquimatica; as celulas das paredes, quando isoladas, são arredondadas, de 5,67—13,23 u de diametro.

Ascos apedicelados, aparafisados, elipsoides, evanescentes, bisporos, 44,8—50,4 × 30,8—36,4 u.

Ascosporos cilindro-elipticos, de polos arredondados, feofragmos, com 5 celulas, constritos nos septos, 25,46—45,36 × 12,23—18,90 u.

Isolado sôbre *purpura* — Malvacea, indet.

Mat. bot. nos 1350 e 1351 e p. m. 1095, 1096, 1097, 1098 e 1099.

Dimeriellopsis araliae n. sp.

Meliolaceas

Micelio negro, tenue, efuso, superficial, epifilo, revestindo parcial ou, totalmente, a epiderme das folhas. Hifas vegetativas septadas, não dematioides, sem hifopodios; ausencia de setas. As celulas hifais têm de 10—25 × 5—7,5 u.

Peritecios globosos, sesseis, esparsos, ornados de setas, não ostiolados; coloração negra, á lupa, e consistencia carnosa; estrutura das paredes periteciais parenquimatica, formada por uma só camada de celulas poligonais, de

5—10 u de diametro. Os peritecios medem de 110—175 u de diametro; as suas setas variam de 50—112,5 de comprimento, tendo a largura de 5—7,5 u; na sua maioria são continuas, apresentando-se porém uma ou outra como septadas, fuligineas e rijas. Fig. 43 (Pl. XXXVIII)

Ascos cilindraceos ou clavados, pouco numerosos, de disposição basal-umbelada, apedicelados, aparafisados, 50—72,5 × 10,15 u. Fig. 44 (Pl. XXXVIII).

Ascosporos cilindro-fusoides, monosticos, obliquos, triseptados, hialinos, 20—25 × 5—6,5 u.

Picnidios ausentes.

Ocurrencia de *Netrocymbe Lafoensiae* n. sp. verificada como associação.

Esta especie foi isolada sôbre "croton tapa muro"-*Polyscias gnifoylei* Baylei e *Bougainvillea sp.* em simbiose com *Orthezia insignis* Douglas-Dois Irmãos. Mat. tipo. no 1169 e 1170 e p.m. 796, 797.

Mycelium nigrum, effusum, epiphyllum ex hyphis rectis; cellula 10—25 × 5—7,5 u.

Perithecia globosa, 110—175 u diam, nigra, carnosa, sessilia, sparsa, parietibus ex cellulis polygonalis, 5—10 u diam. Setis perithecialis simplicis, continuis vel septatis, 50—112,5 × 5—7,5 u.

Ascis cylindraceis vel clavatis, apedicellatis, aparaphysatis, 50—72,5 × 10—15 u.

Ascosporis cylindro-fusoidis, monostichis, triseptatis, hyalinus, 20—25 × 5—6,5 u. Pycnidiis absentis.

In foliis vivis *Polyscias gnifoylei* Bailey Typus nos 1169 et 1170 in Secção de Fitopatologia-Instituto de Pesquisas Agronomicas-Brasil.

Tonduzia fuscata n. sp.

Meliolaceas

Subiculo negro, escasso, superficial, epifilo, formando maculas aqui e ali. Hifas vegetativas fuscas, não dematioides, pouco ramificadas, mas intercruzadas, com celulas de 12,5—22,5 × 5—7,5 u.

Ausencia de setas e de hifopodios. Peritecios glabros, negros, esfericos ou globosos, sesseis, repousando no subiculo, esparsos, apresentando-se de colapso deprimido, á maturidade, 156—195 u. Paredes parenquimaticas, constituidas por varias camadas de celulas poligonais, de 2,5—7,5 u de diametro; consistencia membranosa; ostiolo regularmente não destacado. Fig. 45 (Pl. XXXVIII).

Ascos originados em agrupamento basal-umbelado, lunados, apedicelados, aparafisados, numerosos, encerrando 4 esporos em regra, 50,4—72,5 × 16,8—19,6. Fig. 46 (Pl. XXXIX).

Ascosporos hialinos, septados, aciculares, curvados, do tipo escolecosporo, arrumados uns ao lado dos outros, nos ascos e tendo o mesmo comprimento destes, 50,4—72,5 × 4,2—5 u.

Picnidios ausentes. Triposporium presentes.

Isolado sôbre Araçaseiro, *Psidium aracá.* Raddi em simbiose com *Orthezia insignis* Douglas. Tambem em associação com êsse fungo, foi assinalado *Trichomerium psidii* n. sp.

A especie ora criada está representada pelo tipo de no 1177 e 1178 que se conserva na Secção de Fitopatologia do I.P.A. e p.m. 801, 802, 803, 804, 805, 806, 807.

Localidade tipo: Monteiro. Recife. Pernambuco.

Isolado tambem sobre coatinga de macaco *Calopogonium vellutinum* Benth, 1186.

Subiculum nigrum, in plagulas epiphyllas; hyphis rectis, non constrictis, obscure brunneis rectangulariter ramosum, ex cellula 12,5—22,5 × 5—7,5 u. Perithecia membranosa, glabra, nigra, espherica vel gobosa, sessilia, 156—195 u diam, ad maturitatem collabentibus, ostiolo minuta; parietibus ex cellulis polygonalis, — 2,5—7,5 u diam.

Ascis lunatis, apedicellatis, quadrisporis, aparaphysatis, numerosis, 50,4—72,5 × 16,8—19,6 u.

Ascosporis septatis, hyalinis, acicularis, curvatis, 50,4—72,5 × 4,2—5 u.

Pycnidiis absentis.

In foliis vivis *Psidium aracá* Raddi, in symbiose cum *Orthezia insignis* Douglas. Typus no 1177 et 1178, in Secção de Fitopatologia-Instituto de Pesquisas Agronomicas-Brasil.

Ophiomeliola mangiferae n. sp.

Meliolaceas

Subiculo escasso, radiante, limitado a uma pequena area em que se situam os peritecios, de coloração fusca. Hifas vegetativas não constritas, quasi nada ramificadas e intercruzadas, como que constituindo reticulo, tendo celulas de 14—16,8 × 5,6—8,4 u, em geral. Algumas dessas hifas se apresentam bastante compridas, retilineas, fuscas, setiformes, com a extremidade mais clara e obtusa, indivisas, chegando até 252 × 5,6 u; nelas, as celulas se apresentam de maior extensão, de 11,2—22,4 u. Ocorrem tambem, hifopodios, simples, de inicio, sem celula basal, e depois constituindo capitulo, com 28 u de diametro, do qual se originam novas hifas vegetativas.

Disposição superficial, epifila, não relacionada com os estomatos das folhas.

Peritecios globosos ou esfericos, 154—210 u de ostiolo diferençado nas preparações microscopicas; sesseis, glabros, esparsos, de colapso deprimido á maturidade. Paredes compostas de x camadas de celulas poligonais e arredondadas, 8,4—14 u de diametro; consistencia membranosa; coloração chocolate. Fig. 47 (Pl. XXXIX).

Ascos fusoide—oblongos, Fig. 48, octosporos, pedicelados e parafisados, numerosos, constituidos em agrupamento basal-umbelado, 42—56 × 11,2—14 u. pedicelo de 14 u de extensão; parafises com a porção terminal bifurcada.

Ascosporos fusoide-filiformes, escolecosporos, arrumados uns sobre os outros, de polos agudos, hialinos durante muito tempo, mais tarde olivaceos, de 36,4—42 × 1,8—2,8, pluriseptados.

Picnidios ausentes. Triposporium presente.

Em associação com *Trichomerium psidii* n. sp., vivendo igualmente, em simbiose com *Orthezia insignis* Douglas.

A especie tipo obedece aos no 1179 e 1180 e p. m. 807—811 — tendo sido isolada sobre mangueira-*Mangifera indica* L.

Subiculum in plagulas epiphylas, fusco-nigras, tenues. Mycelium radiatum, rectangulariter ramosum, 5,6—8,4 u diam. Hyphis setiformibus rectiusculis, fuscis, simplicis, 252 × 5,6 cum cellulis 11,2—22,4 u × 5,6—8,4 u. Hyphopodiis primo simplicis dein capitulatis, 28 u diam.

Peritheciis globosis, ostiolatis, sessilis, collabentibus ad maturitatem, membranaceis, brunneis, 154—210 u diam; parietibus ex cellulis poligonalis, 8,4—14 u compositis.

Ascis octosporis, oblongo-fusoidis, 42—56 × 11,2—14 u — curto pedicellatis, 14 u; paraphysis ad apicem bifurcatem, Ascosporis fusoide, filiformibus, primo hyalinis dein olivaceis, pluriseptatis, 36,4—42 × 1,8—2,8 u.

Pycnidiis absentis; triposporium presentis.

In foliis vivis *Mangifera indica* L, cum *Orthezia insignis* Douglas.

Typus 1179 et 1180 in Secção de Fitopatologia, Instituto de Pesquisas Agronômicas-Brasil.

Ceratospermopsis n. gen.

Meliolaceas

Micelio efuso, superficial, epifilo, não possuindo hifas dematioides e originando subículo, em areas esparsas; hifas vegetativas de ramificação cruciforme, sem hifopodios. Setas presentes, em geral de apice acicular e mais ou menos indivisas.

Hifas conidioforicas produzindo esporos feofragmos, fusoides.

Peritecios globosos ou subglobosos, negros, astomos, glabros, membranosos e parenquimaticos, dispostos sôbre o subiculo.

Ascos pouco numerosos, agrupados á maneira de umbela, subglobosos a cuneiformes, aparafisados, octosporos, polisticos.

Ascosporos cilindro-fusoides, dictiosporos, olivaceos, com apendice hialino em ambos os lados.

Esse genero é criado para conter as especies *C. Cupaniae sp.* e *C. Xylopiae n. sp.* derivando-se o seu nome da similitude apresentada para com o genero *Ceratosperma*, da mesma familia.

Mycelium effusum; ex hyphis rectis, cruciformibus. Setae hyphales simplicis. Cleistothecia globosa, nigra, astoma, glabra, membranosa-parenchymatica.

Ascis sub-globosis vel cuneiformis, aparaphysatis, octosporis. Ascosporis cylindro-fusoidis, muriformibus, cum cillia pollaris.

Ceratospermopsis Xylopiae n. sp.

Meliolaceas

Micelio epifilo, negro, superficial, constituido por hifas direitas, longas, fuscas, ramificadas, ás vezes de maneira cruciforme, tendo células de 10—25 × 5—12,5 u; aqui e ali o micelio adensa-se, intercruzando-se as hifas para a formação de subículo; em geral, o subículo é frouxo e de aspecto reticulado, ao microscopio. Fig. 49 (Pl. XXXIX).

Em áreas esparsas do micelio, observam-se setas que, á primeira vista, lembram picnidios, com 130—175 × 7,5 u; aparentemente, são indivisas, com a extremidade livre acicular. Hifopodios ausentes. Em tôrno do subiculo, ou até mesmo nêle se integrando, aparecem conidioforos, não bem diferençados, produzindo conidios feofragmos, multiseptados, fusoides, de 50—57,5 × 7,5 u.

Esporos triposporio tambem presentes.

Peritecios superficiais, assentes no subículo, globosos, 150—225 u, um tanto deprimidos quando maduros, glabros, ástomos, de estrutura parenquimatica e células poligonais, de 7,5—12,5 u de diametro, libertando os ascos por fragmentação do tôpo; consistencia membranosa e coloração fusca.

Ascos em agrupamento basal umbelado, subglobosos a cuneiformes, sesseis ou curto pedicelados, octosporos, polisticos, aparafisados 37,5—50 × 20—27,5 u. Fig. 50 (Pl. XL).

Ascosporos fusoides, a principio hialofragmos, logo depois muriformes e olivaceos-fuscos, curvos, ornados de apendices hialinos em ambas as extremidades; têm x septos transversais e dois ou três longitudinais, retos ou obliquos; medem 20—27,5×7,5—10 u, sem incluir o tamanho dos apendices. Fig. 51.

Essa especie foi isolada sobre Imbira vermelha — *Xylopia frutescens* e o material tipo, sob o qual a elegemos, obedece ao no 1267, no laboratorio da Secção de Fitopatologia do I.P.A. e p. m. de nos 951 a 959 e 976 a 977. Apipucos — Recife.

Assinalado tambem sobre ingá da mata, *Inga sp.* — mat. no 1263 S/ Imbiriba da mata — *Scheiwelera luschnatii* mat. no 1272.

Mycelium epiphyllum, nigrum, ex hyhis fuscis, cruciformibus; cellula 10—25 × 5—12,5 u. Setae hyphales simplicis, 130—175 × 7,5 ad basim. Conidiophoris presentis; conidiis pluriseptatis, fuscis, fusoidis, 50—57,5 × 7,5 u. Triposporium presentis. Cleistothecia globosa, 150—225 u — diam, fusca, astoma, glabra, parenchymatica, membranosa; parietibus ex cellulis polygonalis, 7,5—12,5 diam.

Ascis subglobosis vel cuneiformis, curto-pedicellatis, octosporis, polystichis, aparaphysatis, 37,5—50 × 20—27,5 u.

Ascosporis fusoidis, ab initio pluriseptatis, hyalinis, dein muriformibus, olivaceo-fuscis, cilliatis, 20—27,5 × 7,5—10 u.

Ad folia viva *Xylopia frutescens* et *Inga sp*, et *Schweilera luschnatii.*

Typus 1267 in Secção de Fitopatologia — Instituto de Pesquisas Agronômicas-Pernambuco. Brasil.

Ceratospermopsis Cupaniae n. sp.

Meliolaceas

Micelio efuso, negro, superficial, epifilo, de hifas direitas, fuscas, longas, compondo subículo frouxo, aqui e ali e de aspecto reticulado; hifas ramificadas á maneira de cruz, sem hifopodios e possuindo células de 10—25 × 5—10 u. Setas esparsas, de — 112—170 × 12,5 u na base, de septação não destacada. Conidioforos presentes, produzindo, conidios feofragmos, fusoides, multiseptados de 47,5—55 × 7,5 u.

Peritecios sub-globosos, 215,5—325 u, negros, astomos, glabros, membranosos, de estrutura parenquimatica, superficiais, mas, assentes no subículo. Fig. 52 (Pl. XL).

Ascos em disposição basal umbelada, elipticos a cuneiformes, octosporos, polisticos, aparafisados 47,5—62,5 × 25—32,5, sesseis ou curto pedicelados.

Ascosporos cilindro fusoides ou mesmo fusoides, geralmente incurvados, hialofragmos quando jovens e feodictos depois de maduros, com um apendice hialino em cada polo; medem 37,5—52,5 × 7,5—10 u.

Essa especie é muito próxima do *C. Xylopiae n. sp.*, diferindo dela, entretanto, pelas proporções dos corpos frutigenos e estruturas esporologicas.

Ha sido encontrada sôbre Camboatan de leite *Cupania sp.*, em Apipucos, Recife.

O tipo obedece ao no 1282 e p.m. 992 e 993 do herbario da Secção de Fitopatologia, do I.P.A.

Mycelium effusum, epiphyllum, nigrum; ex hyphis rectis, cruciformibus; cellula 10—25 × 5—10 u. Setae hyphales simplicis 112—170 × 12,5 u ad basim. Conidiophoris presentis; conidia pluriseptatis, fuscis, 47,5—55 × 7,5. Cleistothecia sub globosa, 215,5—325 u diam, nigra, astoma, glabra, membranosa, parenchymatica.

Ascis elipticis vel cuneiformis, octosporis, polystichis, aparaphysatis, 47,5-62,5 × 25,5—32,5 u. Ascosporis cylindro-fusoidis, recurvis, ab initio pluri septatis, hyalinis, dein muriformibus, fuscis, cilliatis, 37,5—52,5 × 7,5—10 u.

Ad folia viva *Cupania sp.*

Typus 1282 in Secção de Fitopatologia-Instituto de Pesquisas Agronômicas-Pernambuco-Brasil.

Leptomeliola puberula n. sp.

Meliolaceas

Micelio negro, epifilo, efuso, sem recobrir, contudo, o limbo foliar; superficial, aparentemente reticulado, de hifas direitas, intercruzadas, fuscas, com celulas de 12,5—20 × 7,5—10 u.

Hifopodios presentes, Fig. 53, elipticos ou globosos, alternos, bicelulares em regra, com a celula terminal de 10—15 × 7,5—15 u, sendo a celula basal cilindrica, na maioria das vezes. Setas igualmente presentes no micelio, erectas, indivisas, direitas, fuligineas, de apice obtuso, chegando a altura de 300 u; as cellulas das setas variam de 32,5—45 × 7,5—10 u.

Cleistotecios globosos, fuscos, astomos, de estrutura parenquimatica e consistencia membranosa, com uma só camada de celulas poligonais, de 3,75—10 u de diametro; isolados, esparsos, setosos, apresentando setas cilindricas, fuligineas, indivisas, até 162,5 u de altura, um tanto curvadas, com celulas de 17,5—22,5 × 5,0 na base. A' maturidade, os peritecios são um tanto deprimidos; medem de 150—212,5. Fig. 55 (Pl. XLI).

Ascos clavados, numerosos, em agrupamento basal umbelado, sesseis, parafisados, disticos a polisticos, 47,5—57,5 × 20—25 u. Fig. 54.

Ascosporos fusoides, multiseptados, feofrágmos sem apendices, 25—32,5 × 5,0—7,5 u.

Estagio imperfeito ignorado.

A especie em apreço é descrita como nova, estando o tipo representado pelo material sobre o qual foi isolado — *Vismia sp* — lacre.

Mat. herb. no 1277 e p.m. 980, 981, 982, 983 e 984, tendo como localidade tipo Apipucos.

Mycelium effusum, nigrum, epiphyllum; ex hyphis rectis, fuscis, cellulis 12,5—20 × 7,5—10 u. Hyphopodia elliptica vel globosa, alterna, bicellularibus, cellula terminalis 10—15 × 7,5—15 u. Setae hyphales rectis, 300 u, long. septatis, cellulis 32,5—45 × 7,5—10 u.

Setis cleistothecialis rectis vel recurvis, simplicis, septatis, 17,5—22,5 × 5 u, ad basim; 162,5 u longae. Cleistothecia globosa, fusca, astoma, membranosa, parenchymatica, sparsa, collabentibus, 150—212,5 u diam.

Ascis clavatis, numerosis, apedicellatis, paraphysatis, 47,5—57,5 × 20—25 u. Ascosporis fusoidis, pluriseptatis, fuscis, 25—32,5 × 5—7,5 u.

Ad folia viva *Vismia sp.*

Typus 1277 in Secção de Fitopatologia-Instituto de Pesquisas Agronomicas-Pernambuco. Brasil.

Tricothyriopsis alineum n. sp.

Tricothyriaceas

Micelio superficial, relativamente escasso, formando membrana brilhante, de coloração negra; em geral epifilo, mas aparecendo como anfigeno, em algumas folhas. Hifas dematioides ou uma ou outra retilinea, fuscas, com celulas de 7,5—15 × 5—7,5 u.

Setas do micelio septadas e constritas, sinuosas ou mesmo incurvadas, tendo de 47,5—85 u de altura; as suas celulas possuem de 5—12,5 u na maior extensão, ou seja no sentido longitudinal.

Peritecios arredondados, achatados algumas vezes, com a região basal invertida, constituindo o topo, recobertos por trama subiculoide, esparsos, ostiolo indistinto, 62,5—92,5 u de diametro; consistencia membranosa, transparente, notando-se os ascos, ao microscopio, sem dificuldade; coloração negra, quando — vistos á lupa e amarelo laranja ao microscopio, montados em azul de Aman. Setosos, com setas identicas ás do micelio; hipostroma ausente; celulas das paredes periteciais arredondadas, de 2,5—7,5 u. Fig. 56 (Pl. XLI).

Ascos eliptico-fusoides, apedicelados, aparafisados, octosporos, polisticos, 40—47,5 × 20—27,5 u. em agrupamento umbelado, pouco abundantes.

Ascosporos em cone truncado, hialofragmos, com 3—6 septos, constritos, 22,5—27,5 × 7,5 u.

Picnidios cilindricos, de paredes densas, apresentando — bico fimbriado, negro-brilhantes, numerosos, 75—112,5 × 25—30 u. Fig. 57 (Pl. XLI).

Picnidiosporos hialodidimos, fusoides, de celulas aparentemente iguais, 12,5—15 × 2,5—3,75.

Foi isolado esse fungo em associação com o micelio de *Trichomerium psidii n. sp.*

Sobre folhas de Resedá — *Lawsonia inermis L.* A especie tipo obedece ao no 1253 e p.m. 945, 946, 947, 948, no herbario da Secção de Fitopatologia, do I.P.A.

Mycelium epiphyllum vel amphigenum, membranaceum, nigrum; ex hyphis dematioidis vel rectilineis, fuscis, cellulis 7,5—15 × 5—7,5 u.

Setae hyphales simplices, septatis, constrictis, 47,5—85 u longae, ex cellulae 5—12,5 u longae. Perithecia globosa, 62,5—92,5 u membranosa, nigra. Setis perithecialis conformibus; parietibus ex cellulis globosis, 2,5—7,5 u.

Ascis elliptico-fusoidis, apedicellatis, aparaphysatis, octosporis, 40—47,5 × 20—27,5 u. Ascosporis hyalinis, pluriseptatis, constrictis, 22,5—27,5 × 7,5 u.

Spermogonia cylindrica, nigra, numerosa, ad apicem fimbriata, 75—112,5 × 25—30 u.

Sporidis fusoidis, hyalinis, bicellularis, 75—112,5 × 25—30 u.

Ad folia *Lawsonia inermis* L. Typus 1253 in Secção de Fitopatologia-Instituto de Pesquisas Agronômicas-Pernambuco-Brasil.

Asterina solanicoloides, var. atypica (Rehm) n. var.

Microthyriaceas

Micelio epifilo, compondo plágulas orbiculares, de 1—5 mms de diametro; mais frequentemente, porem, essa plagulas se tornam confluentes, perdendo a sua delimitação.

Hifas fuscas, de ramificação retangular ou oposta, septadas, formadas por células de 15—22,5 × 5—7,5 u. Setas ausentes.

Hifopodios alternos, como ligeiras intumescencias hemisfericas, unicelulares, 11,2—14 × 8,4—11,2 u, o que constitui caracter diverso do da especie *A. solanicoloides*, em que essas estruturas são de menor proporção e bicelulares.

Tiriotecios sesseis, globoso-deprimidos, desprovidos de setas, de contexto parenquimatico e consistencia membranosa, assentados regularmente sôbre o micelio e medindo de 150—200 u. de diametro; ostiolo abrindo-se em fenda estrelada, á maturidade e bordos laciniados. Fig. 58 (Pl. XLII).

Ascos subglobosos, evanescentes, apedicelados, aparafisados, polisticos, 44,8—52 × 36,4—39,2 u, octosporos.

Ascosporos oblongos a sub ovalados, didimosporos, constritos no septo, com uma celula maior do que a outra, hialinos a principio, depois fuscos, de polos arredondados, 20—25 × 9—12 u. Fig. 59 (Pl. XLII).

A celula maior, mede de 11,2—19,6 × 7—9,8 e a menor de 6—7,5 × 7—9,8 u.

Isolado sôbre oití da praia-*Moquillea tomentosa* (Warm); mat. tipo no 436 e p.m. 999 e 1000.

Essa combinação que ora fazemos destina-se ao ajustamento de nossa especie á descrição de Rehm, pela coincidencia das proporções das estruturas esporologicas e variação no tipo dos hifopodios, ao lado da ausencia de setas periteciais.

O suscetivel da primeira é Solanacea enquanto o da segunda uma Rosacea.

Mycelium epiphyllum, in plagulas orbicularibus 1—5 mms. diam. sparsa vel confluentibus; ex hyphis fuscis, rectangulariter ramificatis, septatis; cellula 15—22,5 × 5—7,5 u. Hyphopodia alterna, unicellularibus, 11,2—14 × 8,4—11,2 u. Tyriothecia sessilia, globoso depressa, membranosa, parenchymatica, 150—200 u diam; ostiolo nitidulo. Ascis sub-globosis, apedicellatis, aparaphysatis, polystichis, octosporis, 44,8—52 × 36,4—39,2 u.

Ascosporis ovoidis vel oblongis, bicellularis, constritis, fuscis, 20—25 × 9—12 u.

Ad folia viva *Moquillea tomentosa* Warm. Typus 436 in Secção de Fitopatologia — Instituto de Pesquisas Agronômicas-Pernambuco-Brasil.

Asterina subinermis Syd

Microthyriaceas

Micelio epifilo, raramente, hipofilo, formando plagulas de 1—5 mm. arredondadas, isoladas, mais tarde confluentes. Hifas fuscas, longas, ramosas, escassamente septadas, com celulas de 10—20 × 3,75—6,25 u. Hifopodios pouco abundantes, continuos, arredondados ou ovalados, ás vezes incurvados, alternos ou mais frequentemente opostos, de 10—12,5 × 5—7,5 u. Setas ausentes, não obstante o caracter filamentoso do micelio, emprestado pela ramificação oposta de suas hifas.

Tiriotecios superficiais, sem hipostroma, radiados desde os bordos, laciniados, ástomos, com himenio simples; forma dimidiada, circular, 175—230 u; disposição gregaria, sob frouxa trama de hifas. Fig. 60 (Pl. XLII).

Ascos globosos ou subglobosos, evanescentes, octosporos, aparafisados, 42,55 × 42,5—45 u, apresentando-se como clavados e longo pedicelados, com pedicelo de 45 × 10 u, quando jovens ainda. Figs 61 e 62 (Pl. XLII—XLIII).

Ascosporos oblongos, feodidimos, com uma celula ligeiramente maior do que a outra, 32,5—37,5 × 12,5—15 u. Fig. 63 (Pl. XLIII).

Esporos triposporium presentes.

A descrição dessa especie é feita segundo Saccardo, pois as dimensões de nosso fungo se enquadram nela, satisfatoriamente.

Foi isolado sôbre folhas de Imbiriba da mata — *Eschweilera luschnatii*, mat. 1281 e p. m. 987, 988, 989, 990, 991.

Dictyopeltis applanata n. sp.

Micropeltaceas — (Dictiopeltinea)

Micelio ausente.

Tiriotecios marron escuro ou fuligineos, astomos, superficiais, livres, glabros, sesseis, de conformação circular, tenues.

Estrutura pletenquimatica, observando-se os ascos atrâvés dela, ao microscopio.

Himenio simples.

Disposição hipofila; diametro de 200—275 u.

Ascos aparafisados, globosos, curto pedicelados, octosporos, polisticos, 15—20 × 12,5—15 u.

Ascosporos do tipo didimosporo, com um segmento transversal, constritos no septo e de fórma fusoide, 10—12,5 × 2,5—3 u, hialinos. Isolado sôbre folhas verdes de sapoti—Achras sapota Mill. — Jardim Zoo Botanico-Dois Irmãos. Recife. Pern.

O material tipo tem o no 1225, na Secção de Fitopatologia, do I.P.A. e p. m. 897 e 898.

Mycelium nullum. Thyriotheca fuliginea, hipophylla, glabra, astoma, circularibus, textura intricata. Hymenio simplici aparaphysato, polyasco. Ascis globosis, pedicellatis, octosporis, 15—20 × 12,5—15 u. Ascosporis bicellularis, constrictis, fusoidis, hyalinis, 10—12,5 × 2,5—3 u.

Ad folia viva *Achras sapota* Mill. Typus 1225, in Secção de Fitopatologia-Instituto de Pesquisas Agronômicas-Pernambuco-Brasil.

Phragmothyriella exserta n. sp.

Micropeltaceas — (Haplopeltineas)

Micelio ausente.

Tiriotecios epifilos, livres, superficiais, não maculicolas, glabros, sesseis; coloração negra; diametro do escutelo de 650—798 u; ostiolo orbicular, definifo, de 20 u de diametro. Fig. 64 (Pl. XLIII).

Estrutura pseudo-parenquimatica, de celulas arredondadas, notando-se raias somente nos bordos do escutelo; essa região é tambem mais clara do que a porção central do ascocarpo, opaca e bem escura.

Himenio simples, poliasco. Ascos sub-clavados, octosporos, disticos, apedicelados, 99—146 × 15—29 u. Fig. 65 (Pl. XLIII).

Parafises presentes, abundantes e filiformes.

Ascosporos em fórma de clava, com dois septos transversais, apresentandose constritos, hialinos, 34—63 × 6—10 u; a celula basal dos ascosporos é a de menor diametro, 4—5 u, porém é a que se apresenta com extensão maior.

Isolado sôbre folhas verdes de louro-*Ocotea* sp.

Jardim Zoo-Botanico, Dois Irmãos. Recife.

O material tipo acha-se na Secção de Fitopatologia do I.P.A., sob no 1226 e p.m. 901, 902, 903 e 904.

Mycelium nullum. Thyriothecia epiphylla, glabra, sessilia, nigra, 650—798 u diam; poro centralis, 20 u diam; textura pseudo-parenchymatica non radiata. Hymenium singulum, polyascum.

Ascis sub-clavatis, apedicellatis, octosporis, 99—146 × 15—29 u. paraphysatis. Ascosporis clavatis, bicellularis, constrictis, hyalinis, 34—63 × 6—10 u; cellula basal longa. Ad folia viva *Ocotea* sp. Typus 1226 in Secção de Fitopatologia-Instituto de Pesquisas Agronômicas-Pernambuco-Brasil.

Phragmothyriella dignota n. sp.

Micropeltaceas — (Haplopeltineas)

Micelio ausente.

Peritecios escutelares, tipo tiriotecio, de bordos raiados, epifilos, glabros, superficiais, sesseis, não maculicolas e esparsos.

As suas caracteristicas á lupa, assemelham-se ás da especie *Phragmothyriella dignota*. Contudo, o diametro dos tiriotecios e o tamanho dos ascos e ascosporos oferecem clara distinção, separando-o daquele fungo.

Himenio simples, poliasco.

Tiriotecios ostiolados, mas com ostiolo em fenda raiada; medem 374—390 u de diametro.

Ascos subclavados ou elipsoides, sesseis, 44,58 × 15—18 u, octosporos, polisticos.

Parafises presentes, abundantes, com 3 a 4 septos ,constritos, hialinos, 19,5—21 × 5 u.

Isolado sobre folhas verdes de louro-*Ocotea sp.* das mesmas peças de onde foi obtido o *P. exserta.*

Mat. tipo de no 1226, na mesma peça de *P. exserta n. sp.* na Secção de Fitopatologia do I.P.A. c. p.m. 899, 850, 851, 852.

Mycelium nullum. Scutellum epiphyllum, glabrum, sparsum, 374—390 u diam, sessilia. Poro apicali nitidulo-radiata. Hymenium singulum, polyascum. Ascis sub-clavatis, sessilis, octosporis, 44—58 × 15—18 u, apedicellatis, paraphysatis. Ascosporis clavatis vel fusoidis, hyalinis, pluriseptatis 19,5—21 × 5 u.

Ad folia viva *Ocotea* sp.

Typus 1226 in Secção de Fitopatologia-Instituto de Pesquisas Agronômicas-Pernambuco-Brasil.

Microthyriella inaequalis n. sp.

Micropeltaceas — (Haplopeltinea)

Micelio ausente.

Tiriotecios livres, superficiais, orbiculares, esparsos, não maculicolas, epifilos, com ostiolo não diferençado, 700—780 u de diametro.

Estrutura parenquimatica, com celulas poligonais, em regra, quadradas, 4—5,2 u.

Himenio simples.

Ascos globosos, sesseis, deitados, aparafisados, octosporos, polisticos, 57,5—80 × 32,5—45 u.

Ascosporos do tipo didimosporo, mono septados, hialinos, sem constrição e aparentemente de celulas desiguais, 37,5—62,4 × 12,5—15 u.

Isolado sôbre folhas verdes de louro-*Ocotea sp.* nas mesmas peças onde se assinalaram *Phragmothyriella exserta* e *P. dignota.*

Material tipo sob no 1226, na Secção de Fitopatologia do I.P.A.

Mycelium nullum. Thyriothecia epiphylla sparsa, 700—780 u diam, textura parenchymatica, parietibus ex cellulis poligonalis, 4—5,2 u; poro centrali, ignoto. Hymenium singulum. Ascis globosis, aparaphysatis octosporis 57,5—80 × 32,5—45 u.

Ascosporis bicellularis, hyalinis, 37,5—62,4 × 12,5—15 u.

Adfolia viva *Ocotea sp.*

Typus 1226 in Secção de Fitopatologia-Instituto de Pesquisas Agronômicas-Pernambuco. Brasil.

Clypeolum circinans Pat.

Micropeltaceas — (Haplopeltinea)

Micelio superficial ausente.

Ascocarpos escutelados, livres, superficiais, negros, esparsos, circulares, glabros, sesseis, sem ostiolo definido, 265—303 u de diametro.

Estrutura pseudo-parenquimatica; himenio simples.

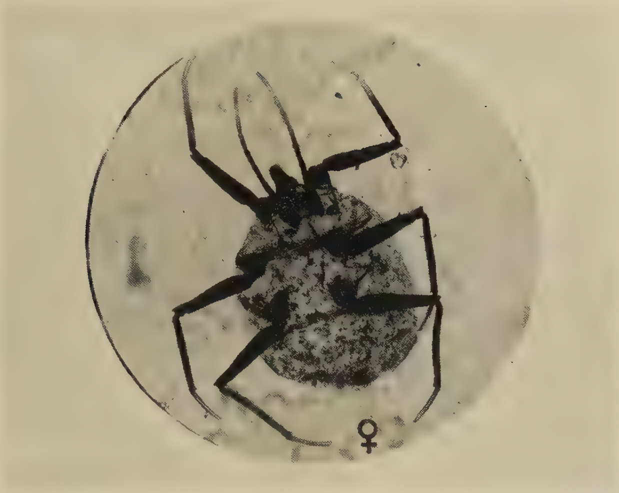
Ascos subglobosos a cilindricos, sesseis, com parafises filiformes, octosporos, polisticos, 38—42 × 14—16 u.

Ascosporos uniseptados, hialinos, de fórma clavada ou oval alongada, 12—15 × 4—5 u.

Não verificamos o caracter circinado dos tiriotecios da especie tipo, mas supomos que se trate do mesmo fungo.

Isolado sobre caule de feijão Guando-*Cajanus indicus Spreng*, tendo sido, anteriormente, encontrado sobre folhas de Asclepiadacea, em S. Jorge, no Equador.

Material herb. sob. no 1193 e p. m. 847.



As figs. 1—5, foram tiradas na Secção de Fitopatologia, pelo colega Ambrosio di Oliveira Freitas.

Fig. 1. *Orthezia insignis Douglas.* Aumento: 48 × (Vista pela parte ventral)

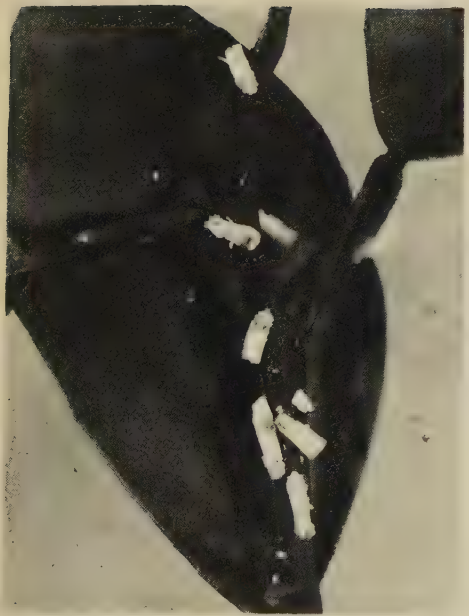


Fig. 2. *Orthezia insignis* Douglas (Larvas e imagos) s/galho de Laranjeira. Aumento: 5 × (*Citrus aurantium L.*)

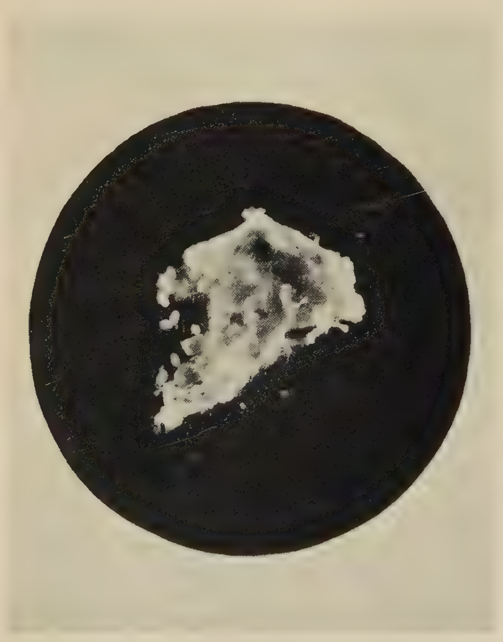


Fig. 3. Ovisaco aberto vendo-se os ovos colados e dispersos. Aumento: 12 ×

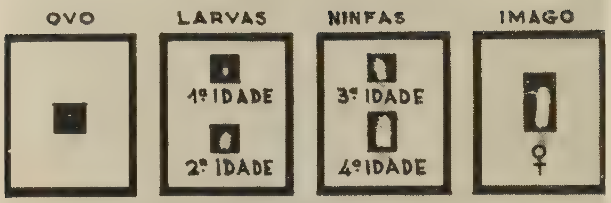

OVO
LARVAS
1ª IDADE
2ª IDADE
NINFAS
3ª IDADE
4ª IDADE
IMAGO
♀

Fig. 4 Ciclo evolutivo da *Orthezia insigni* Douglas

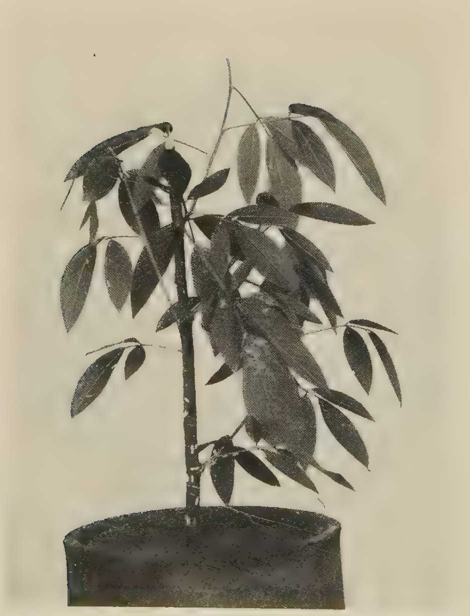


Fig. 5. Feijão guandú (*Cajanus indicus* Spreng) atacado por *Orthezia insignis* Douglas

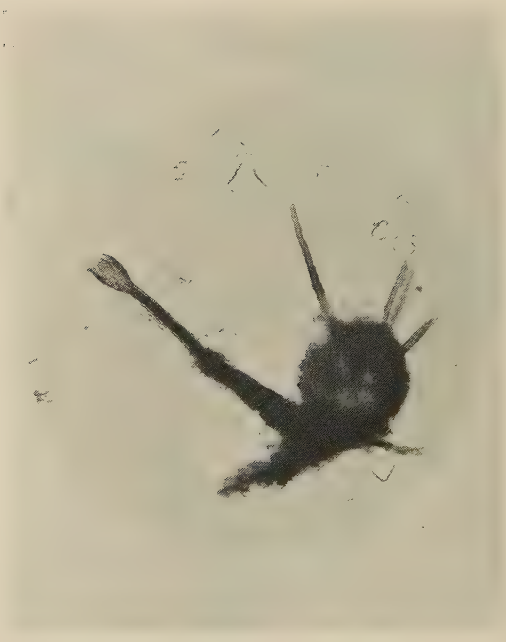


Fig. 6. *Trichomerium crotoni* n. sp. 405 × Peritecio e picnidio

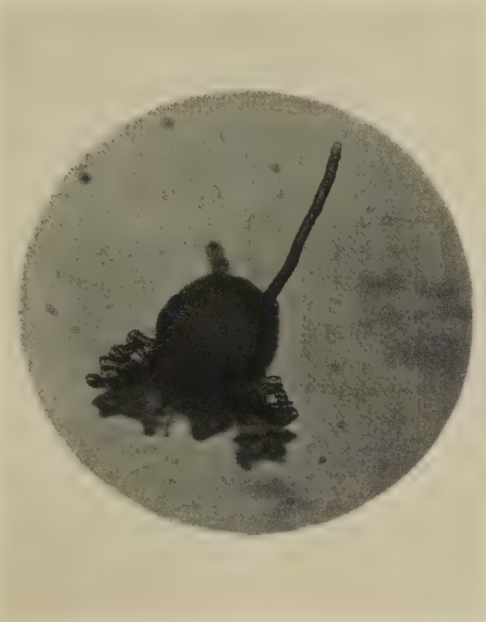


Fig. 7. *Trichomerium crotoni* n. sp. 405 ×
Peritecio.

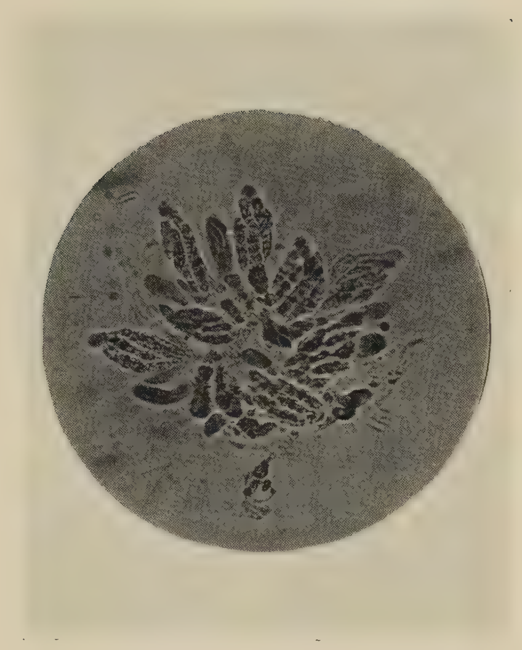


Fig. 8. *Trichomerium crotoni* n. sp. 405 ×
Ascos.

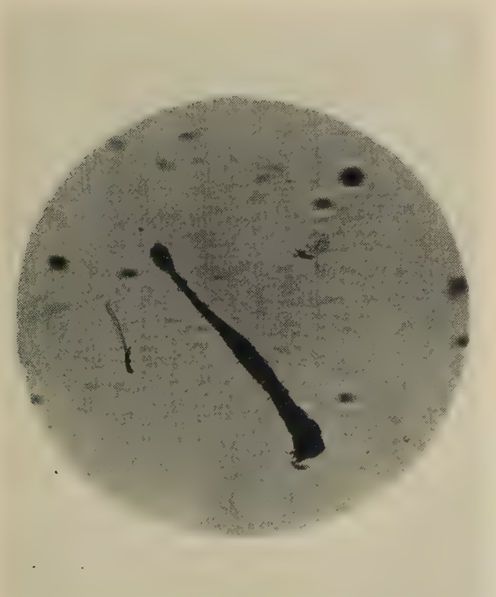


Fig. 9. *Trichomerium crotoni* n. sp. 96 ×
Picnidio.

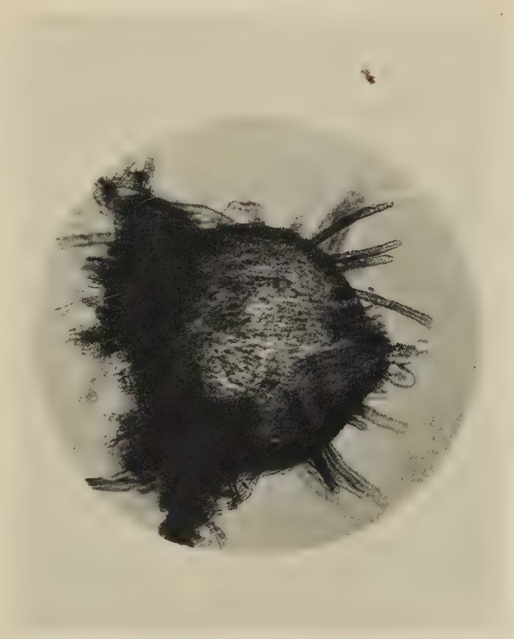


Fig. 10. *Trichomerium psidii* n. sp. 405 ×
tecio.

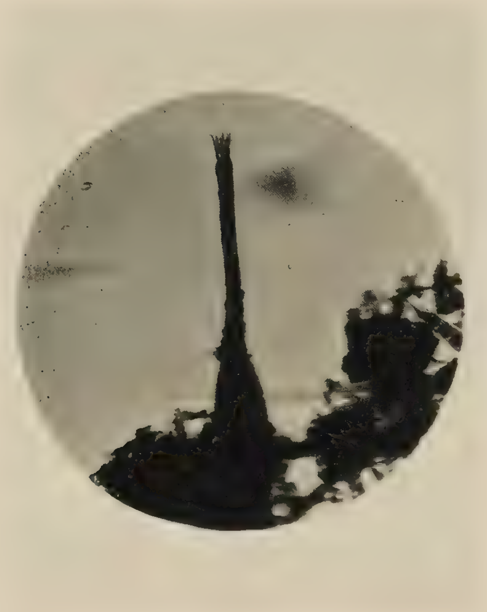


Fig. 11. *Trichomerium psidii* n. sp. 405 ×
Picnidio.

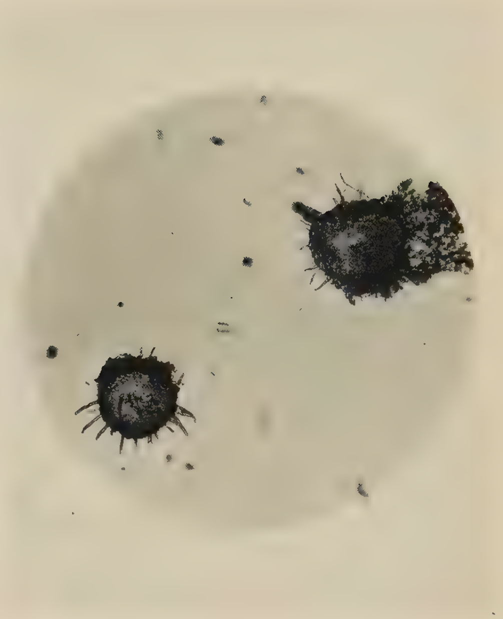


Fig. 12. *Trichomerium hirtellum* n. sp. 72 ×
Peritecios.

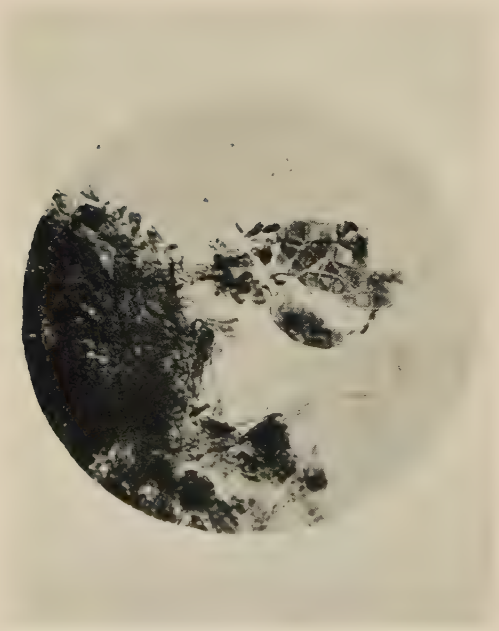


Fig. 13. *Trichomerium hirtellum* n. sp. 405 ×
Ascosporos e ascos.

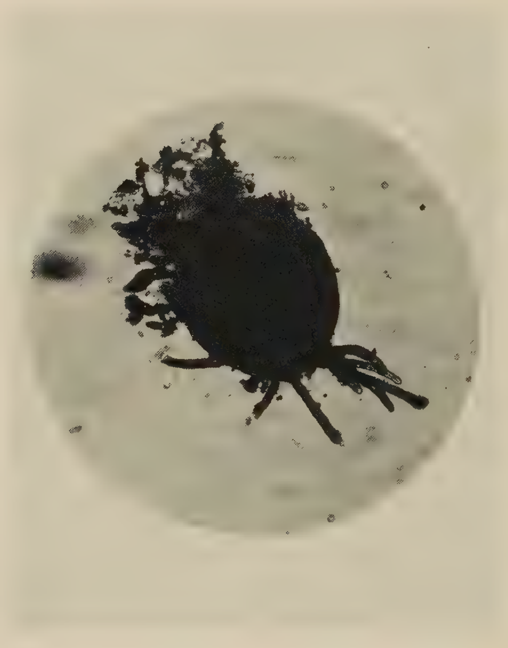


Fig. 14. *Choetopotius commistum* n. sp. 476 ×
Peritecio.

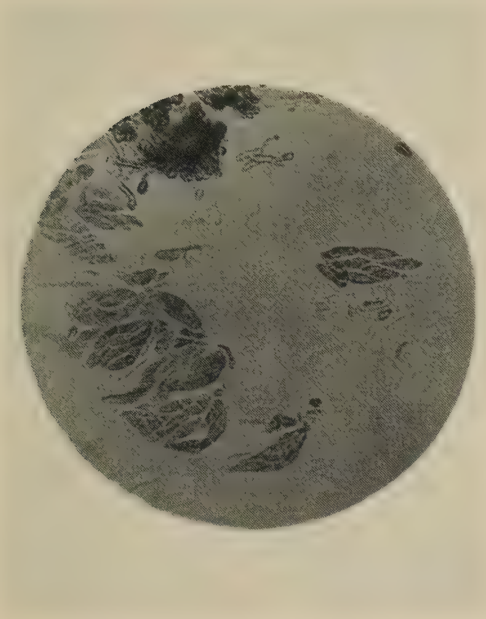


Fig. 15. *Choetopotius commistum* n. sp. 405 ×
Ascos e ascosporos.

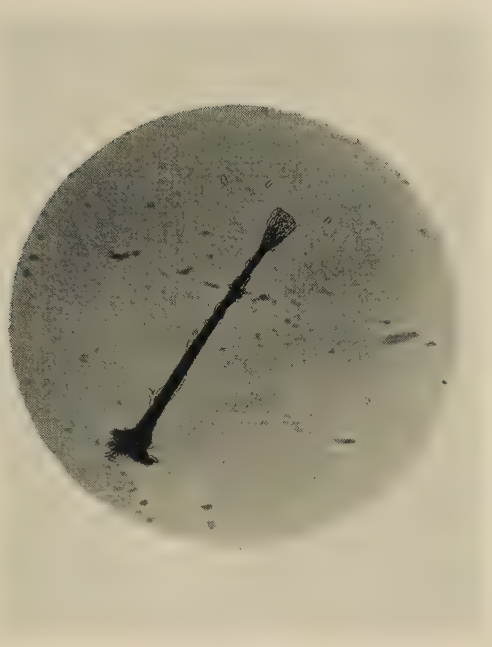


Fig. 16. *Choetopotius commistum* n. sp. *Picnidio* libertando conidios 96 ×

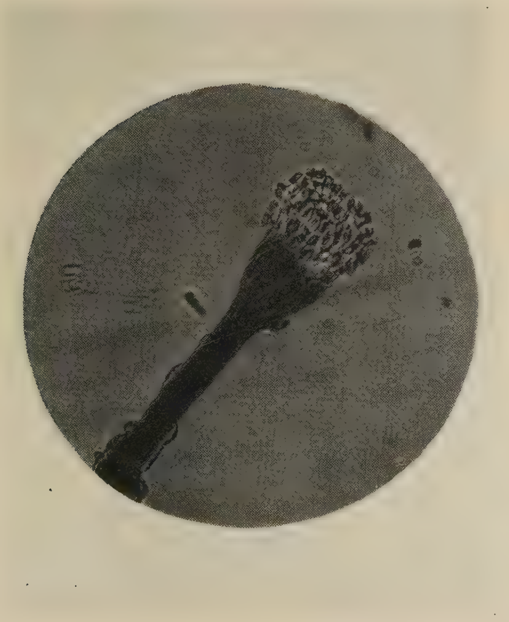


Fig. 17. *Choetopotius commistum* n. sp. *Picnidio-cirro* de conidio 405 ×.

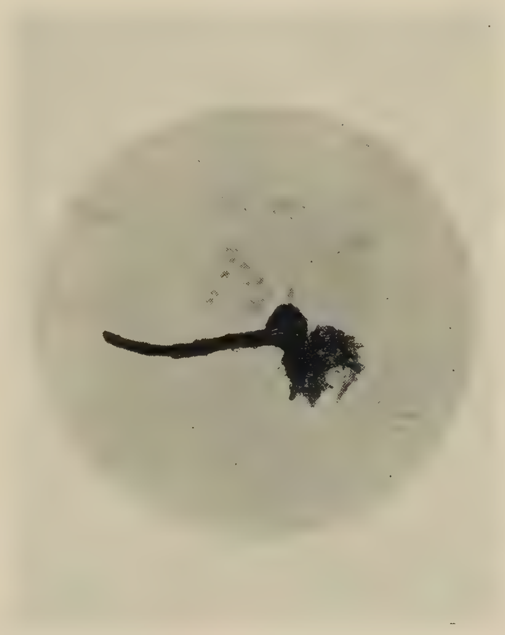


Fig. 18. *Choetopotius commistum* n. sp. 96 ×
Picnidio e peritecio.

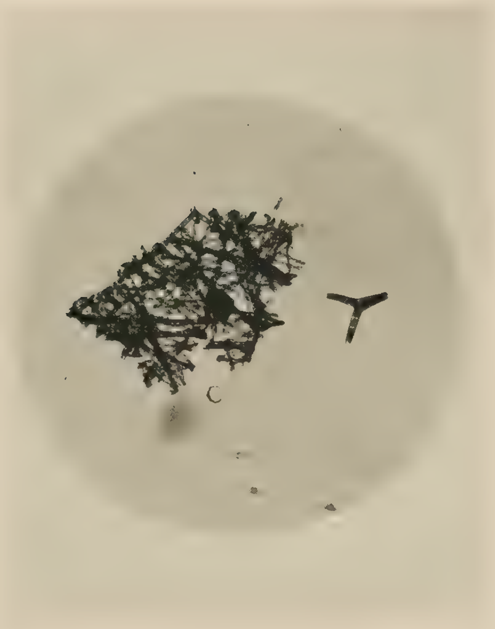


Fig. 19. *Choetopotius commistum* n. sp. 96 × Micelio.

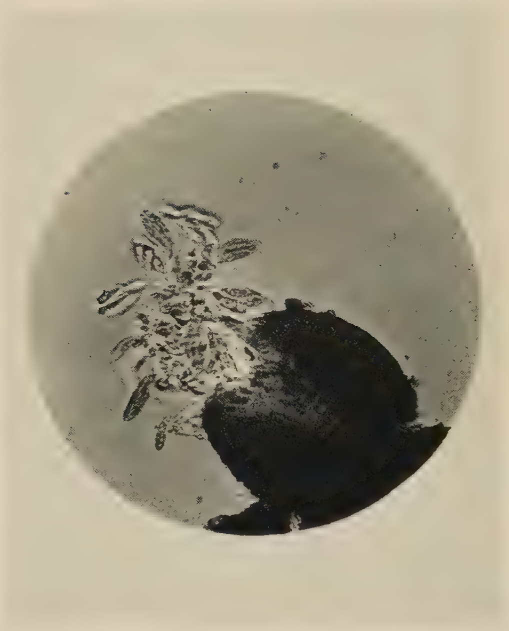


Fig. 20. *Setella coracina* n. sp. 405 × Peritecio e ascos.

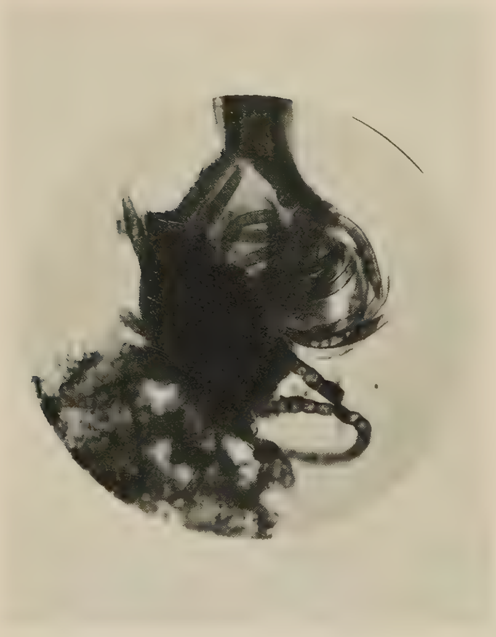


Fig. 21. *Setella coracina* n. sp. 405 × Picnidio fragmentado na base, libertando conidio.

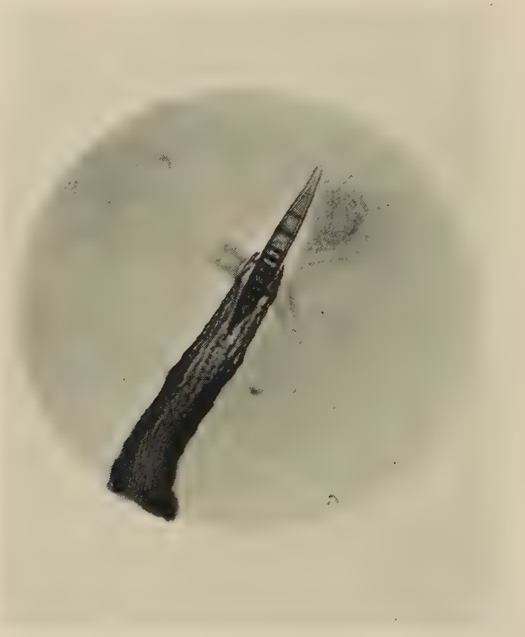


Fig. 22. *Setella coracina* n. sp. 476 × Expulsão de conidio.

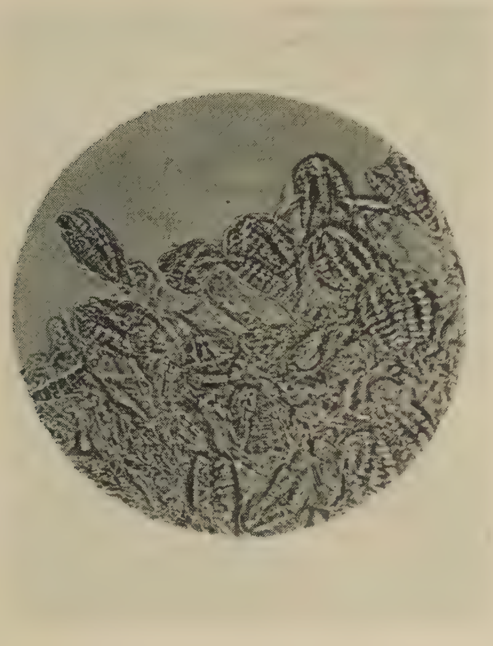


Fig. 23. *Choetomeris hemisphaerica* n. sp. 476 ×
Ascos e ascosporos.

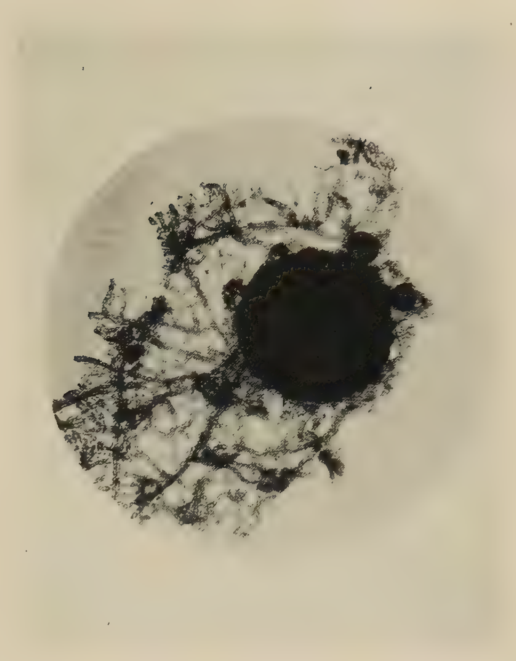


Fig. 24. *Netrocymbe depressum* n. sp. 96 ×
Peritecio e micelio.

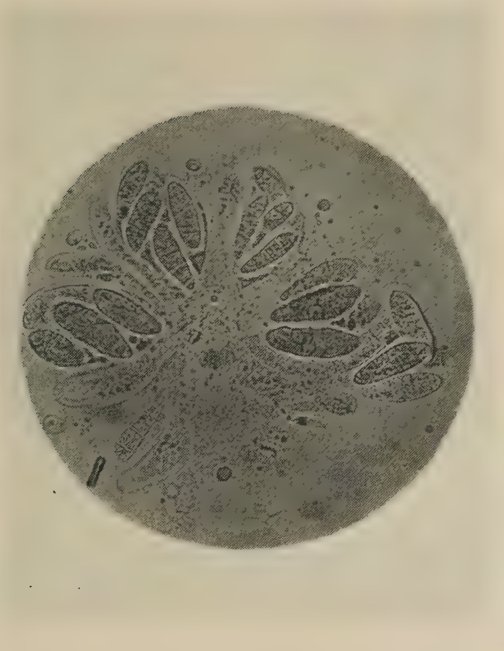


Fig. 25. *Netrocymbe depressum* n. sp. 405 ×
Ascos e ascosporos.

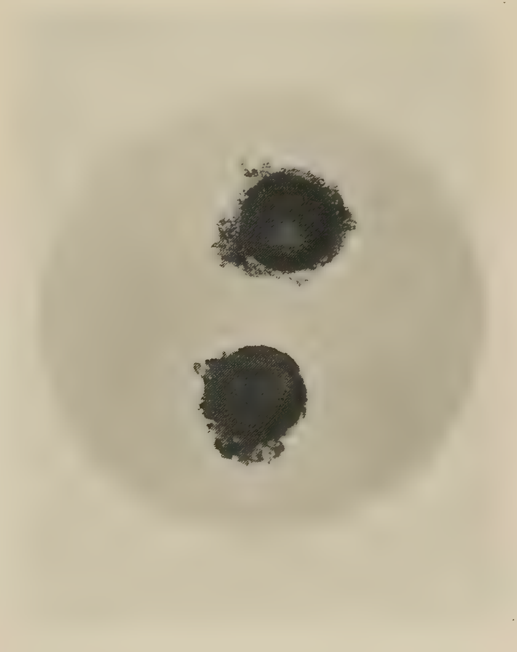


Fig. 26. *Netrocymbe Mauritiae* n. sp. 96 ×
Peritecios.

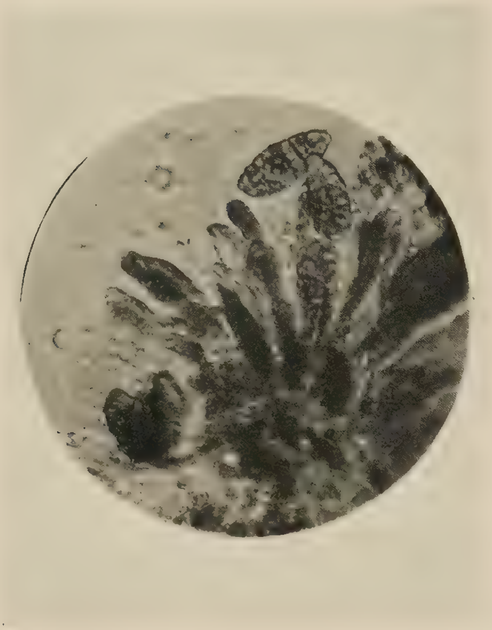


Fig. 27. *Netrocymbe Mauritiae* n. sp. 405 ×
Ascos e ascosporos.

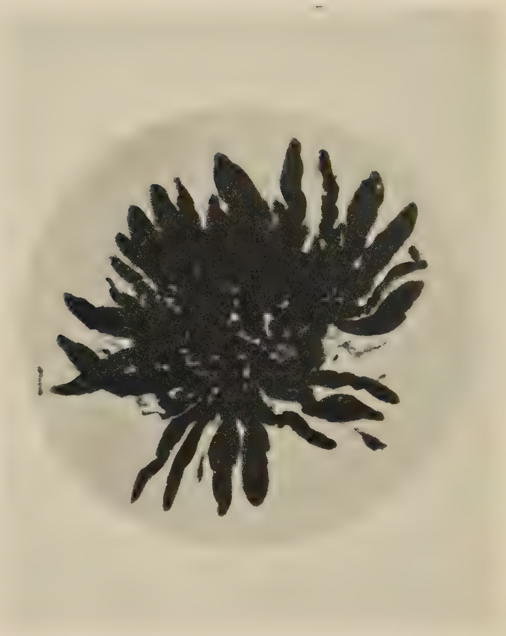


Fig. 28. *Netrocymbe lafoensiae* n. sp. 405 ×
Ascos.

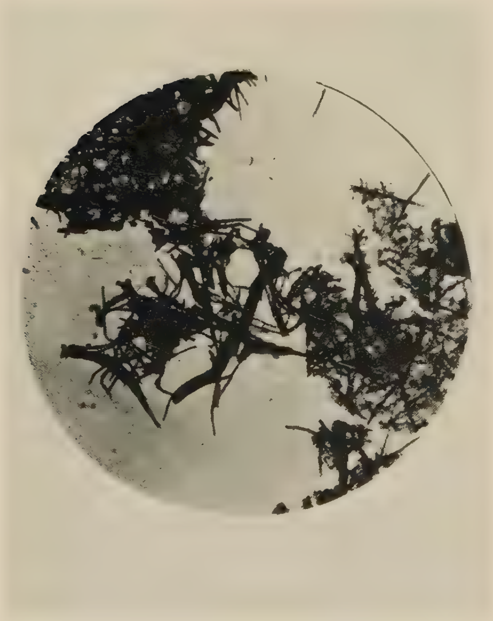


Fig. 29. *Netrocymbe lafoensiae* n. sp. 72 ×
Picnidios.

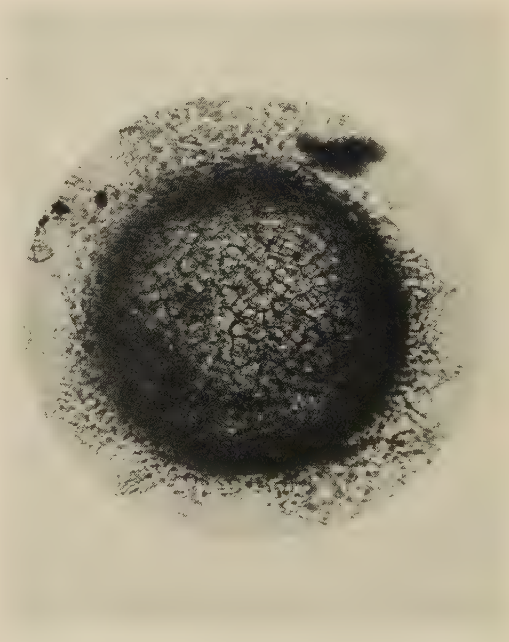


Fig. 30. *Netrocymbe perparum* n. sp. 405 ×
Peritecio.

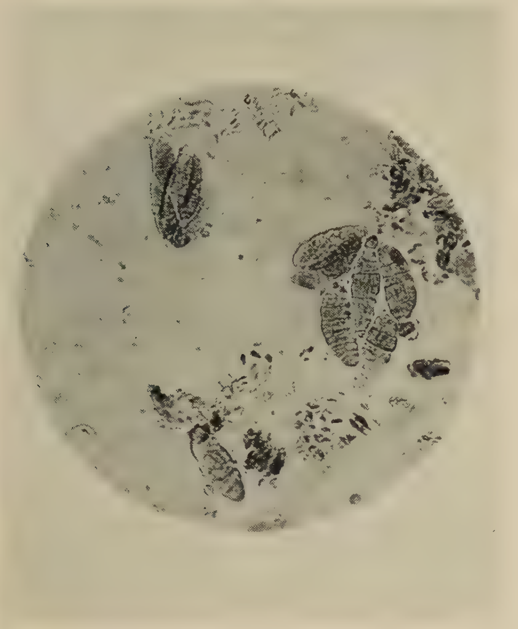


Fig. 31. *Netrocymbe perparum* n. sp. 476 ×
Ascos e ascosporos.

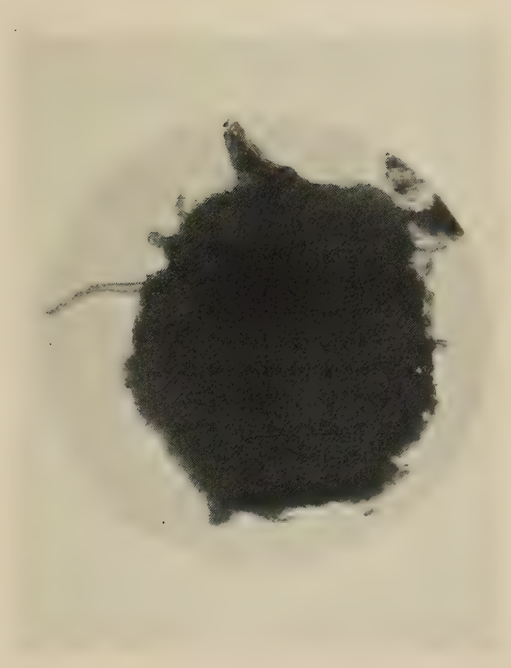


Fig. 32. *Netrocymbe inspersum* n. sp. 476 ×
Peritecio.

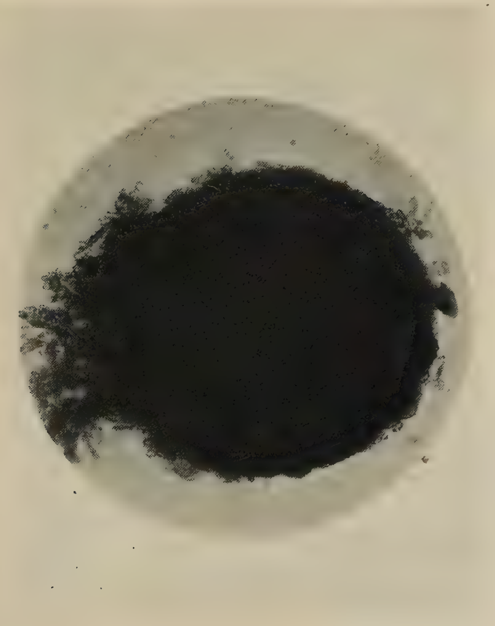


Fig. 33. *Netrocymbe robusta* n. sp. 405 × Peritecio.

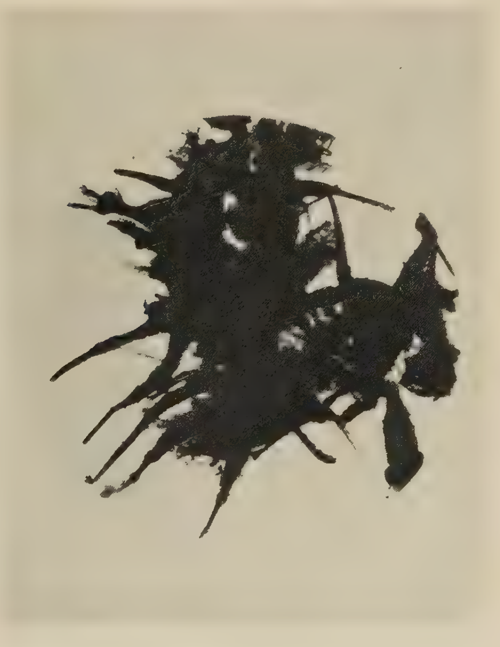


Fig. 34. *Netrocymbe robusta* n. sp. 96 × Picnidios.

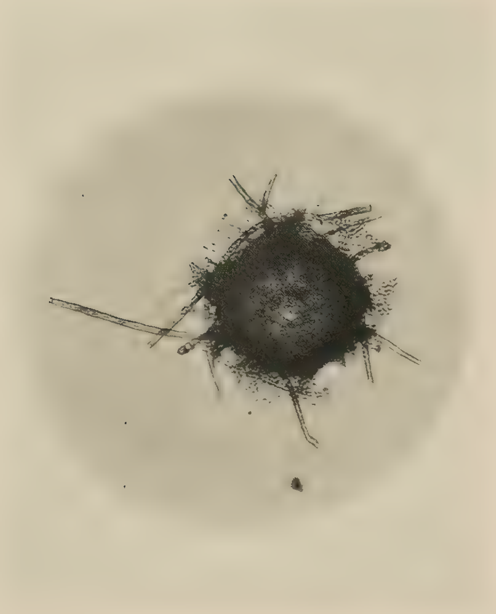


Fig. 35. *Limacinia aurantii* p. Hen. 96 ×. Peritecio.

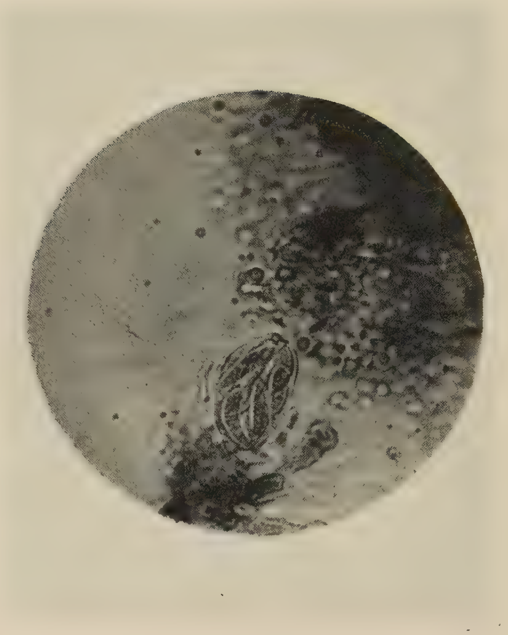


Fig. 36. *Limacinia aurantii* P. Hen. 476 × Ascos e ascosporos

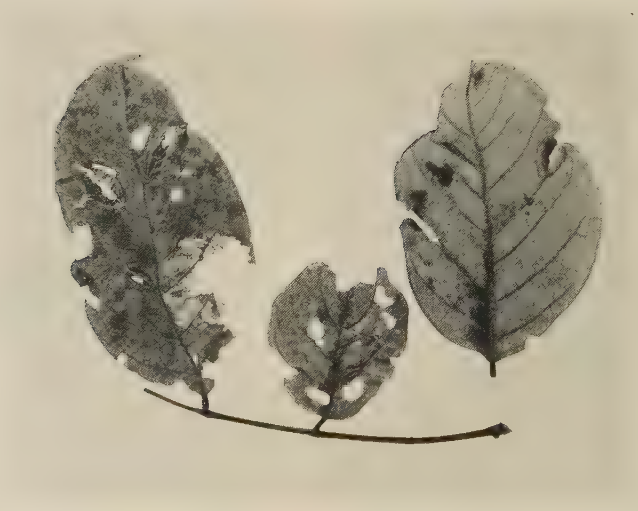


Fig. 37. *Meliola bicornis winter, lonchocarpii* n. sp. S/ *Lonchocarpus sp.*

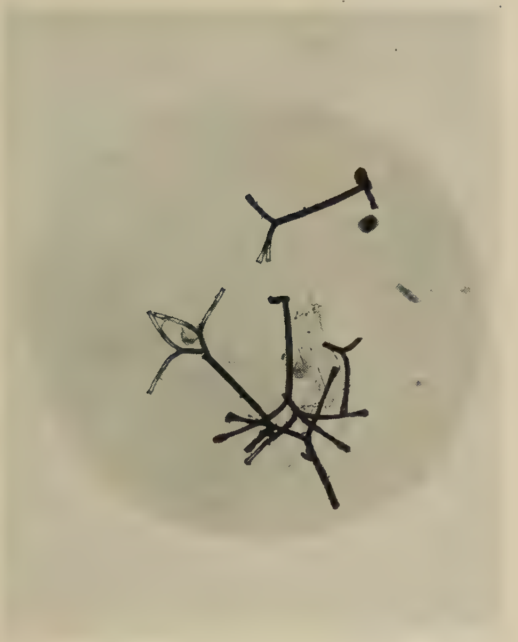


Fig. 38. *Meliola bicornis (Wint) var. lonchocarpii*, n.v. 96 × Setas.

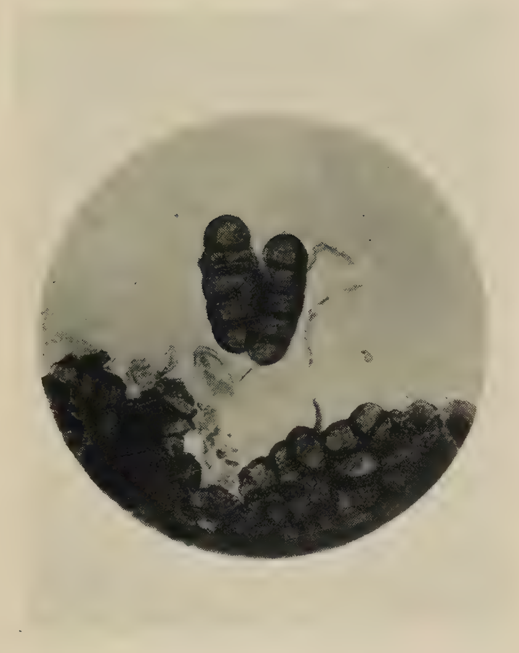


Fig. 39. *Meliola bicornis (Wint) var. lonchocarpii* n.v., 476 × Ascosporos.

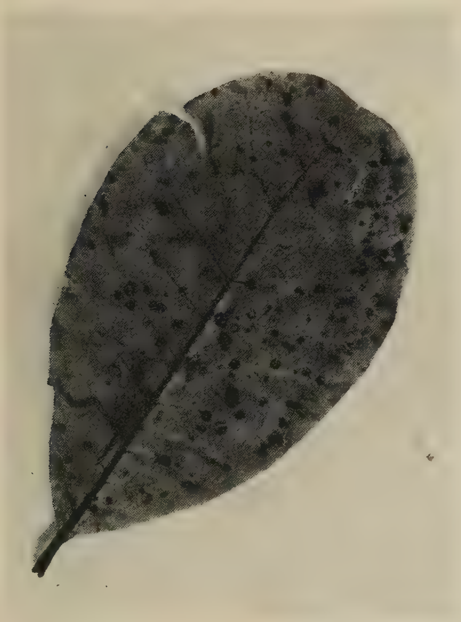


Fig. 40. *Meliola anacardii* A-Zimm S/*Anacardium occidentale* L.

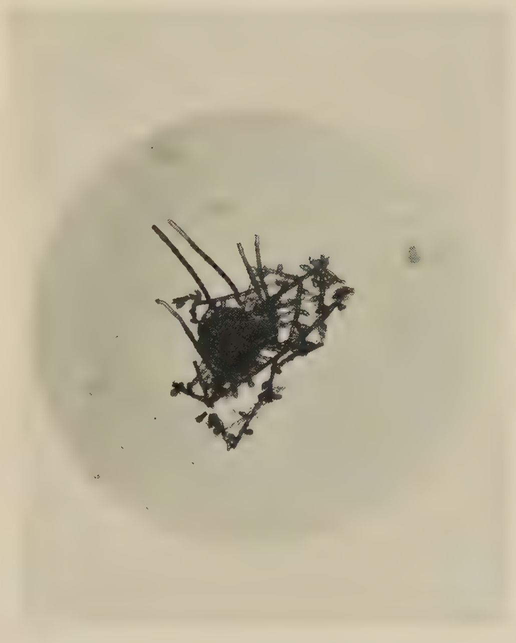


Fig. 41. *Meliola anacardii* A-Zimm 96 × Peritecio e hifopodios.

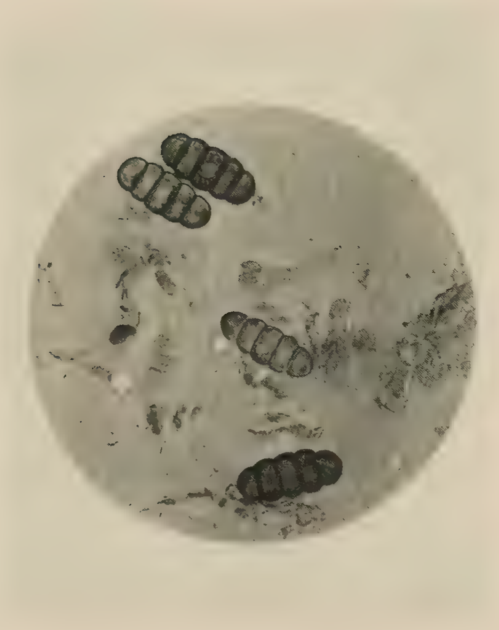


Fig. 42. *Meliola anacardii* A-Zimm. 476 × Ascosporos.

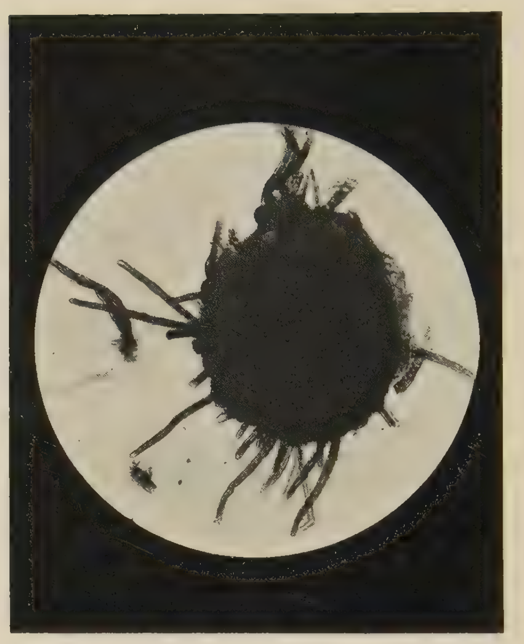


Fig. 43. *Dimeriellopsis araliae* n. sp. 405 × Peritecio.

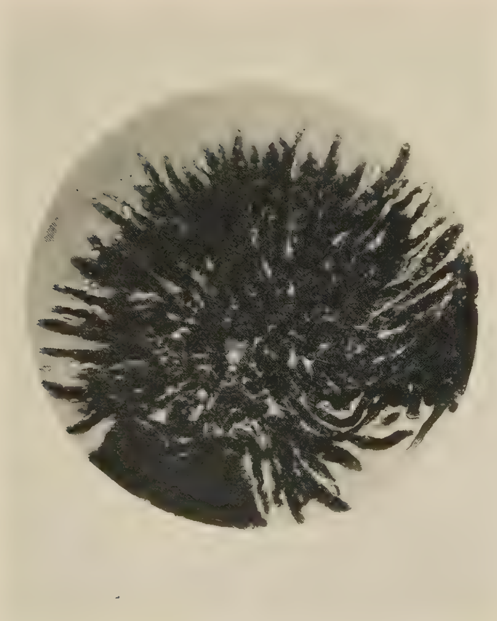


Fig. 44. *Dimeriellopsis araliae* n. sp. 405 × Ascos.

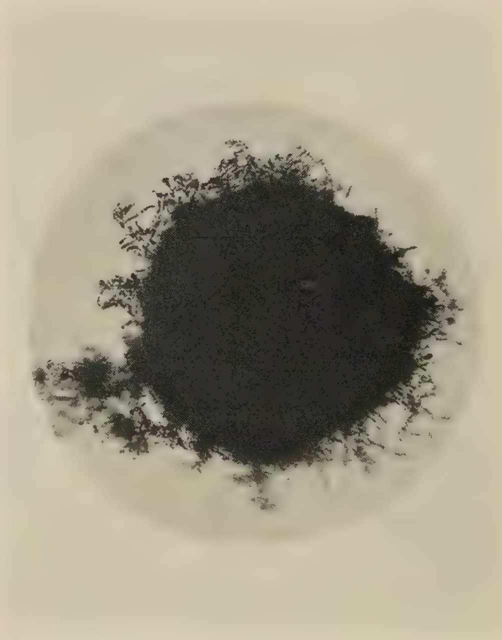


Fig. 45. *Tonduzia fuscata* n. sp. 405 × Peritecio.

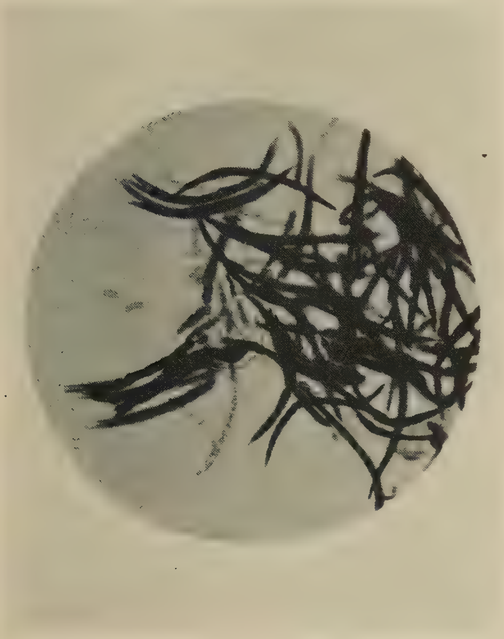


Fig. 46. *Tonduzia fuscata* n. sp. *Ascos* e ascosporos 476 ×.

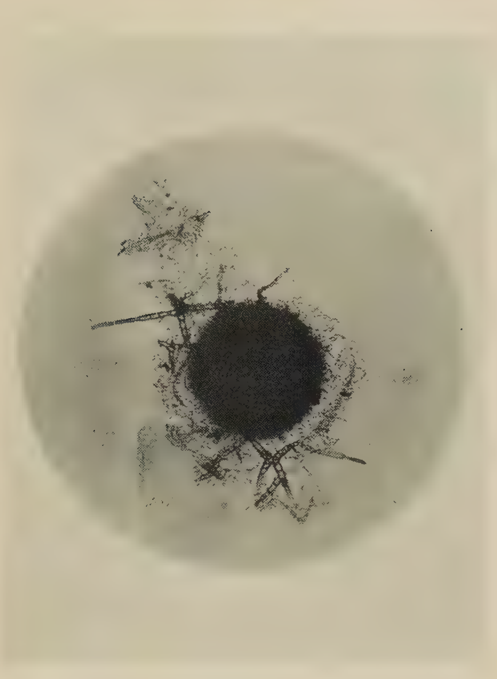


Fig. 47. *Ophiomeliola mangiferae* n. sp. 96 × Peritecio e triposporium.

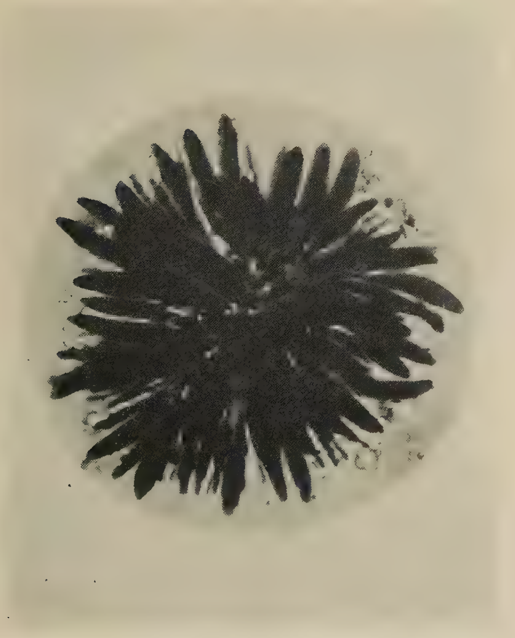


Fig. 48. *Ophiomeliola mangiferoe* n. sp. 405 × Ascos.

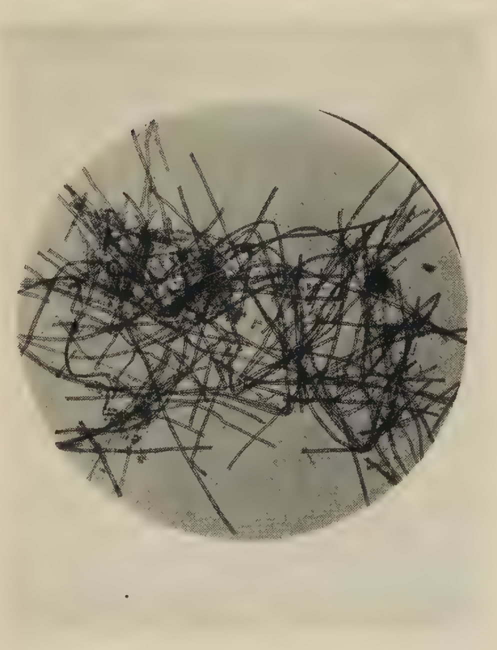


Fig. 49. *Ceratospermopsis xylopiae* n. sp. Hifas do micelio 96 ×.

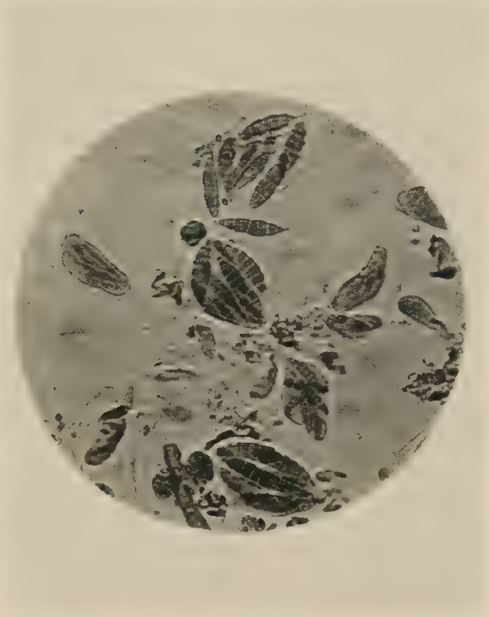


Fig. 50. *Ceratospermopsis xylopiae* n. sp. 476 ×
Ascos e ascosporos.

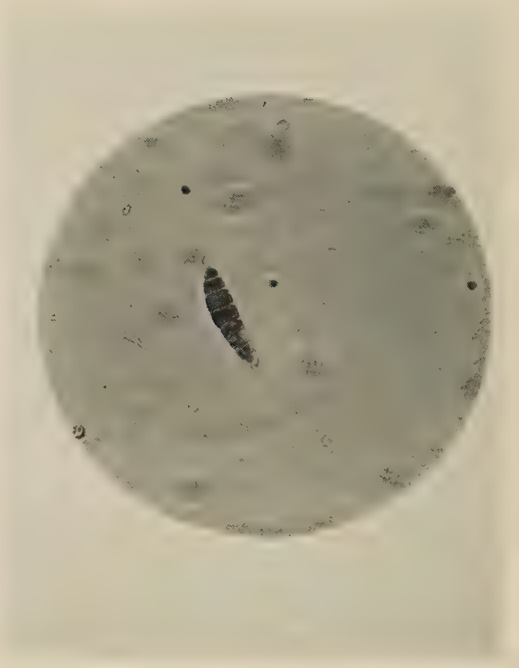


Fig. 51. *Ceratospermopsis xylopiae* n. sp. 476 ×
Ascosporo.

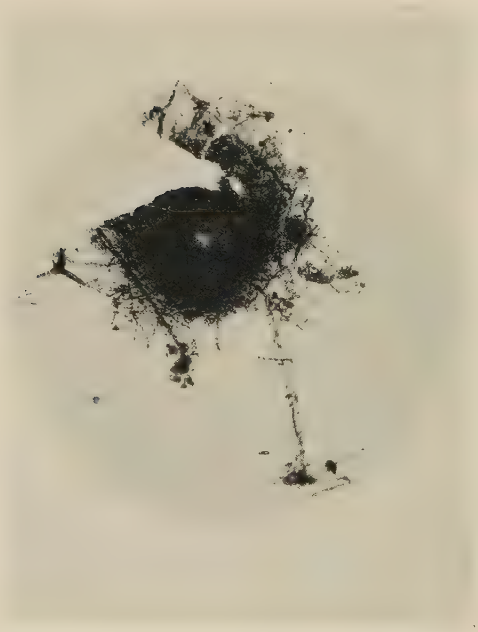


Fig. 52. *Ceratospermopsis cupaniae* n. sp. 96 ×
Peritecios.

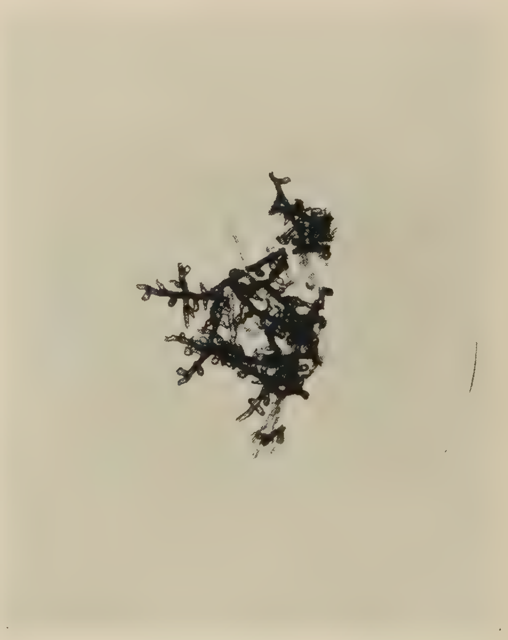


Fig. 53. *Leptomeliola puberula* n. sp. 96 ×
Hifopodios.

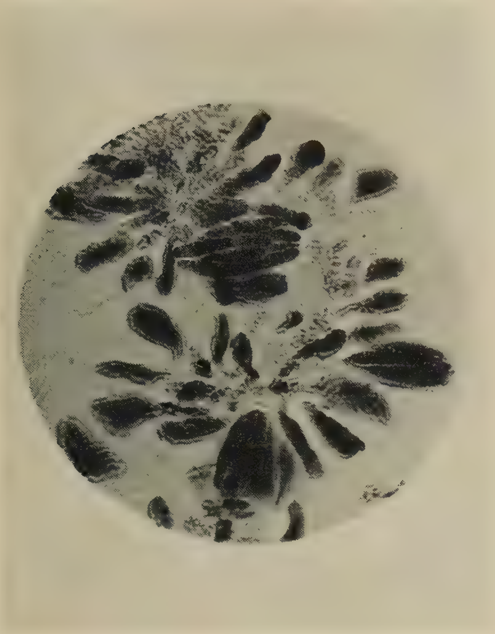


Fig. 54. *Leptomeliola puberula* n. sp. 405 ×
Ascos e ascosporos.

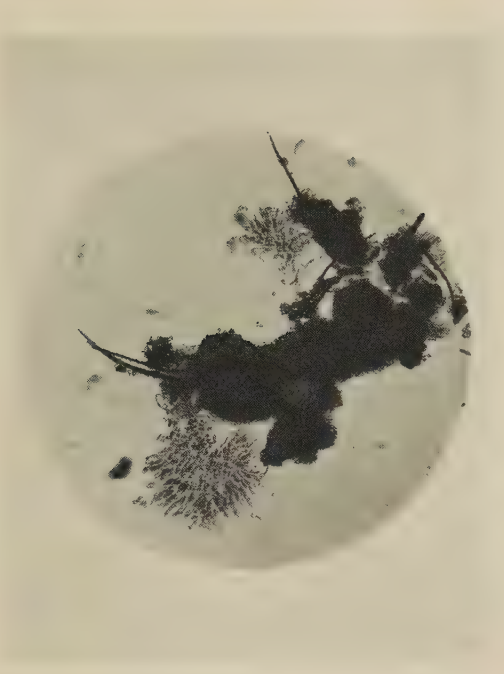


Fig. 55. *Leptomeliola puberula* n. sp. 96 × Peritecio esmagado, notando-se os ascos em liberdade.

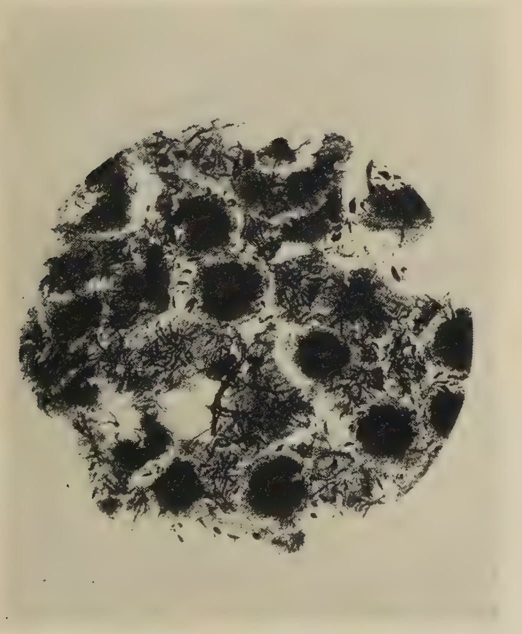


Fig. 56. *Trichothyriopsis alineum* n. sp. 96 ×
Peritecios.

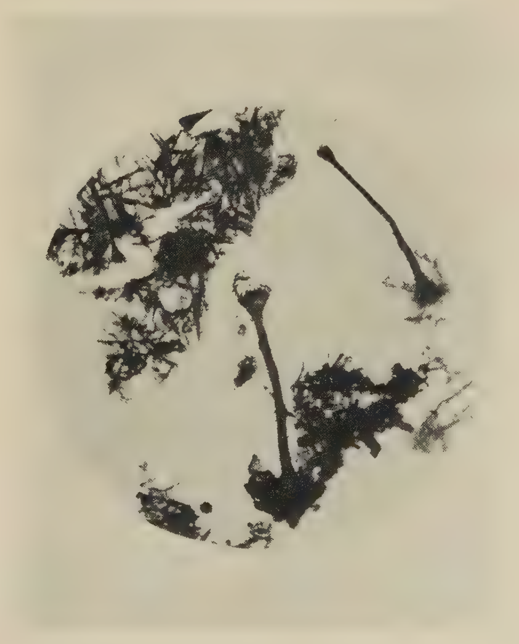


Fig. 57. *Trichothyriopsis alineum* n. sp. 96 ×
Picnidios.

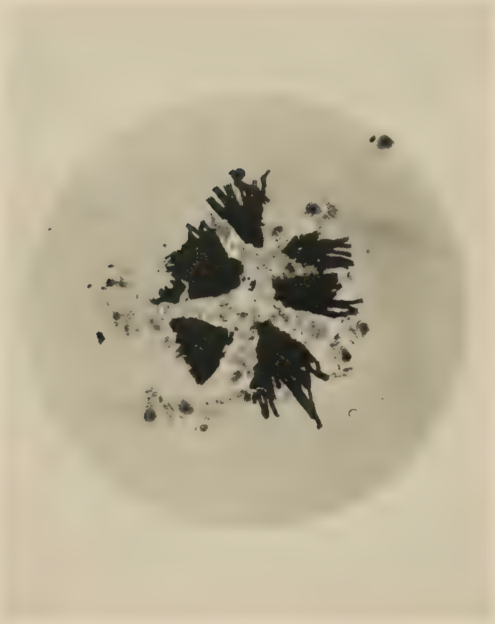


Fig. 58. *Asterina solanicoloides atypica* (*Rehm*) n. var. 96 × Peritecio fragmentado e ascos.

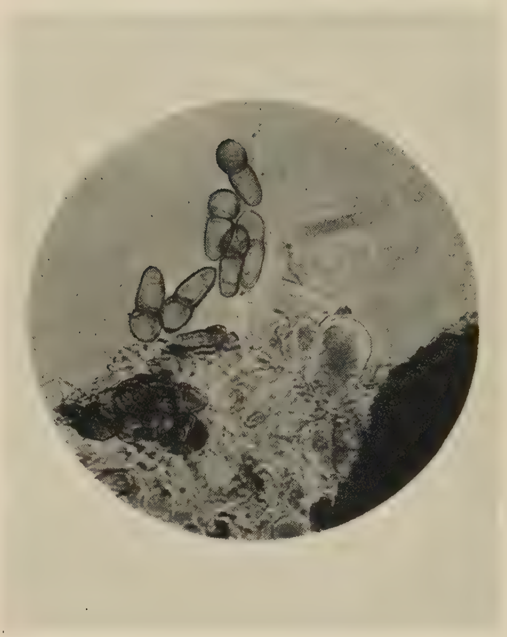


Fig. 59. *Asterina solanicoloides atypica* (*Rehm*) n. var. 476 × Ascosporos.

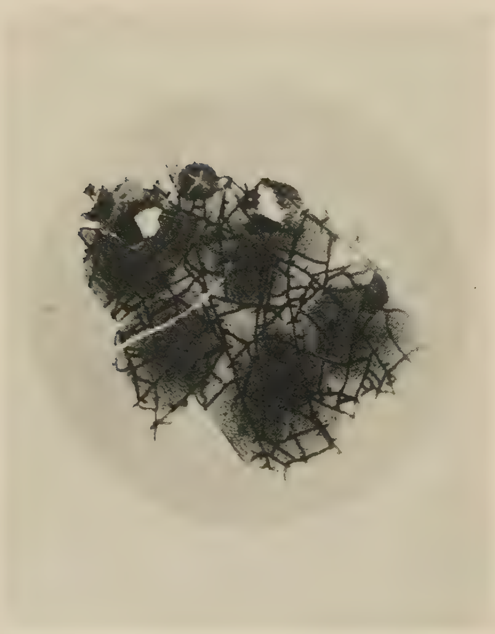


Fig. 60 *Asterina subinermis Syd.* 96 × Tiriotecios.

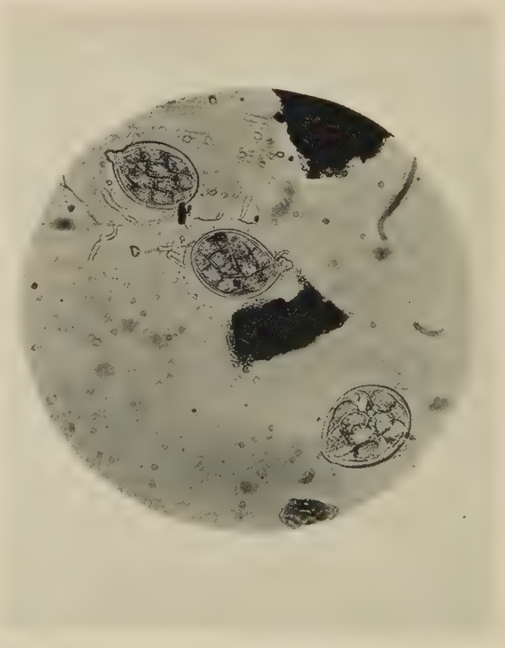


Fig. 61. *Asterina subinermis* Syd 480 × Ascos.

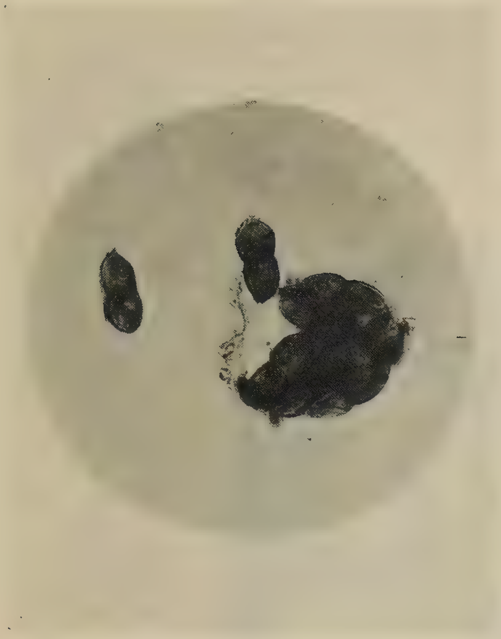


Fig. 62. *Asterina subinermis* Syd 476 × Ascos e ascosporos.

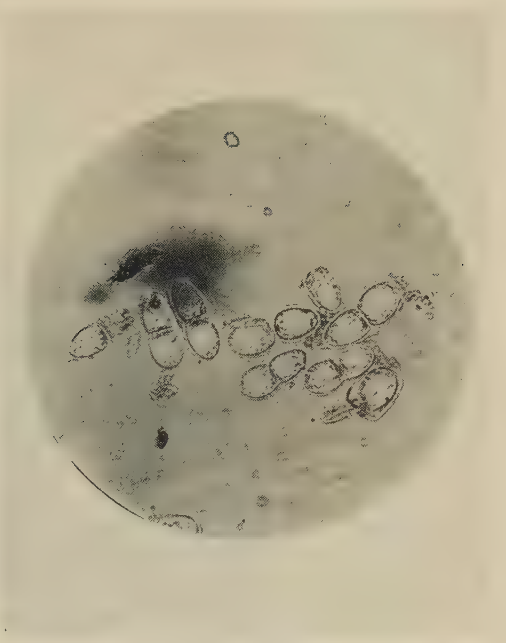


Fig. 63. *Asterina subinermis* Syd. 476 × Ascosporos.

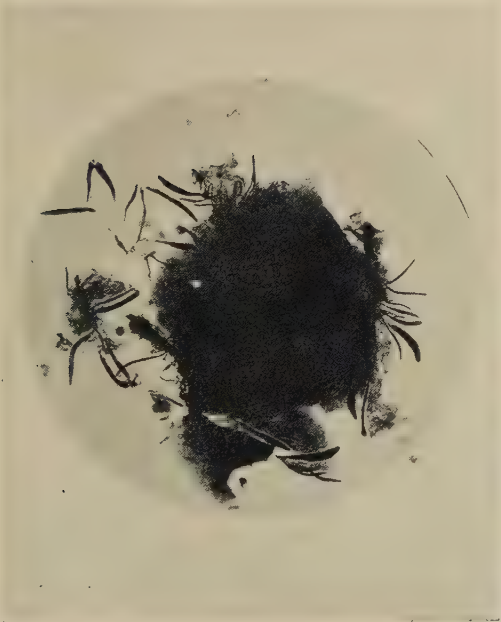


Fig. 64. *Phragmothyriella exserta* n. sp. 96 × Peritecio e ascos.

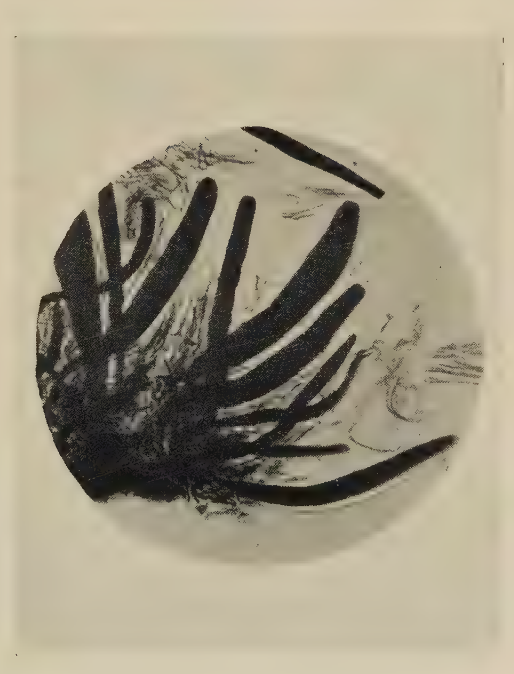


Fig. 65. *Phragmothyriella exserta* n. sp. 450 × Ascos.

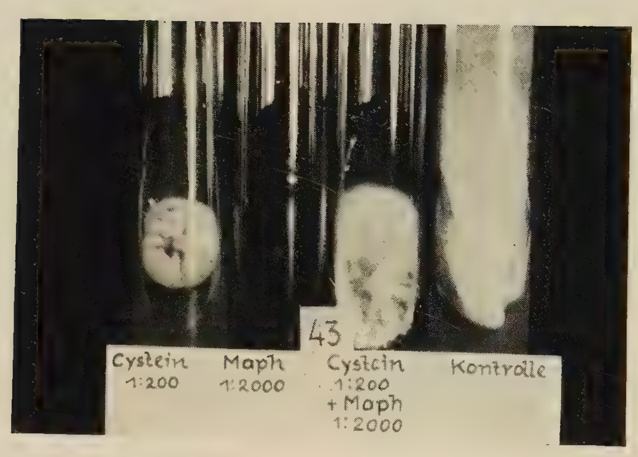

43
Cystein 1:200
Maph 1:2000
Cystein 1:200 + Maph 1:2000
Kontrolle

Abb. 1.

Über die Wirkung schwefelhaltiger Aminosäuren auf das Wachstum verschiedener Fadenpilze „in vitro"

von

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Facharzt für Dermatologie

Über toxische Wirkungen einzelner Aminosäuren auf pflanzliche Organismen insbesondere Bakterien wurde mehrfach berichtet (GLADSTONE, BONNER-TATUM-BEADLE). Bezüglich der hemmenden Wirkung von Aminosäuren auf das Wachstum pathogener Fadenpilze wurde von ROBBINS beobachtet, daß Prolin und in viel stärkerem Ausmass Hydroxyprolin auf Trichophyton mentagrophytes und andere Dermatophyten eine fungistatische Wirkung ausübt. Am empfindlichsten erwies sich ein Stamm von Epidermophyton flocculosum. Zahlreiche nicht pathogene Fadenpilze hingegen, sowie ein Stamm von Rhodotorula blieben von Hydroxyprolin unbeeinflußt.

A) *Versuche über die Wirkung verschiedener in der Haut vorkommender schwefelhaltiger Aminosäuren auf das Wachstum pathogener Pilze.*
Material:

Pilzstämme: Trichophyton mentagrophytes T 669 N
Trichophyton purpureum Bang
Trichophyton interdigitale Priestley
Trichophyton vinosum
Microsporum Audouini
Microsporum lanosum
Cladosporum Wernecki
Hemispora stellata Vuill.
Aspergillus malignus Lindt
Aspergillus Amstelodami Thom und Church

Aminosäuren: 1-Cystein hydrochlorid, 1-Cystin, dl-Methionin, bezogen von der Firma HOFFMANN la Roche in Basel.

Methode: Als Nährmedium wurde CZAPEK-DOX Agar mit Zusatz von Asparagin 1 mg/cm Nährmedium und anstatt Sucrose, Glucose verwendet.[1] Bebrütungstemperatur war 28° C für alle Stämme ausser Aspergillus malignus Lindt, der im Brutschrank bei 35° gehalten wurde. (pH ca 5.5).

Mit den jeweils zu prüfenden Aminosäuren wurden Verdünnungsreihen mit dem geschmolzenen fertigen Nährmedien hergestellt, bei einer Ausgangskonzentration von 200 mgr %, also Verdünnung 1 : 500. Die einzelnen Konzentrationen waren 1 : 500, 1 : 1000, 1 : 2000 und 1 : 4000. In jedes der Röhrehen wurden 10 ccm Nährmedium einpipettiert. Alle Kulturen liefen dreifach. Die Stammkulturen wurden auf dem obenangegebenen Czapek-Dox-Nährboden gezüchtet und von ihnen jeweils ein stecknadelkopfgroßes Mycelstück überimpft. Die Ablesung erfolgte alle 5 Tage, die endgültige Prüfung am 20. Tag.

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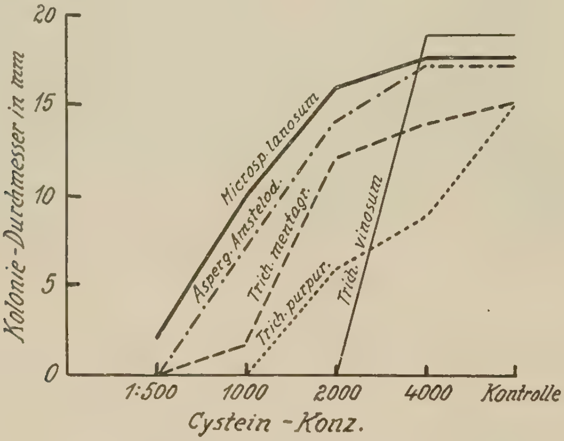
[1] $NaNo_3$ 2,0; KCl 0,5; $MgSO_4$ 0,5. $FeSO_4$ 0,01, KH_2PO_4 1,0 Asparagin 1,0; Glucose 20,0 Agar 20,0 Aqua dest. 1000,0.

Ergebnisse: Während l-Cystin und dl.-Methionin ohne nennenswerten Einfluss auf das Wachstum der geprüften Stämme waren, erwies sich l-Cystein hydrochlorid deutlich hemmend in einer Konzentration von 1 : 2000 aufwärts. Ähnlich wie wir dies bei Fadenpilzen gefunden haben, konnten COOK-PERISUTTI-WALSH einen toxischen Effekt von Cystein in höheren Konzentrationen auf die Respiration der Hefe S. cerivisiae feststellen. Für einige der eigenen Stämme zeigt die beigegebene Tabelle den wachstumshemmenden Effekt höherer Cysteinkonzentrationen.



Obwohl die einzelnen Stämme eine verschiedene Empfindlichkeit aufwiesen, so wurden doch alle mehr oder weniger gehemmt. Eine gewisse Ausnahme bildete Aspergillus malignus Lindt, insoferne als bei diesem Stamm in Konzentrationen von 1 : 500 nur etwa bis zum achten Tag der Bebrütung ein deutlicher Unterschied in den einzelnen Röhrchen erkenntlich war. Später erfolgte überall das gleiche Wachstum. Erst bei Konzentrationen von 1 : 200 und 1 : 100 blieben die weiter unten beschriebenen Wachstumsveränderungen auch bei diesem Stamm bestehen.

Wie Rücküberimpfungen von 30 bis 60 Tage alten Cysteinkulturen auf normales Nährmedium ergaben, hat Cystein keinen fungiziden sondern lediglich einen fungistatischen Effekt.

Bei zwei Stämmen war im Cystein-Nährmedium ausser der Reduktion des Grössenwachstums auch morphologisch eine Veränderung eingetreten. Gladosporium Wernecki zeigt auf normalen Nährboden eine pelzig rauhe Oberfläche von oliv-bis graugrüner Farbe. Am Cystein-Nährboden in einer Konzentration von 1 : 500 unterblieb die Ausbildung von Lufthyphen nahezu vollständig, die Kultur wuchs glatt, feucht und fast schwarz.

Aspergillus malignus Lindt, der zweite Stamm mit morphologischen Veränderungen unter dem Einfluss von Cystein, wuchs in Konzentrationen 1 : 200

bis 1 : 100 in radiären Wülsten, z.T. sogar cerebriform, nahezu ohne ausbildung von Lufthyphen. Ausserdem war die so veränderte Kultur nicht weiß oder cremefarbig wie gewöhnlich, sondern bildete ein rosa bis violettrotes Pigment.

Es ist uns nicht möglich diesen roten Farbstoff chemisch näher zu definieren, jedoch scheint es sich um einen Körper zu handeln der zu seinem Aufbau Schwefel benötigt. Wir beobachteten nämlich in Kontrollversuchen bei Zusatz verschiedener Aminosäuren in Konzentrationen von 1 : 1000 bis 1 : 100, nur noch bei dl-Methionin (1 : 200 und 1 : 100) eine ähnliche Pigmentbildung und diese nur an der agarzugewandten Seite der Kultur. Im übrigen erfolgte aber das Wachstum auf dem methioninhaltigen Nährboden normal. Das ebenso schwefelhaltige l-Cystin liess sich zum Vergleich wegen seiner geringen Löslichkeit nicht heranziehen. An Aminosäuren wurden für diese Kontrollversuche geprüft: l-Lysin, dl-Phenylalanin, l-Alanin, l-Leucin, Glykokoll, dl.-Methionin und Glutaminsäure.

B) *Untersuchungen zum Nachweis des fungistatisch wirksamen Bestandteils im Cystein.*

Für die Aufklärung dieser Wachstumshemmung gab die nahe chemische Verwandtschaft zwischen Cystein und Cystin einen Anhaltspunkt. Da der wesentliche Unterschied der genannten Aminosäuren darin besteht, daß im Cystein der Schwefel in Sulphhydrilform (-SH), im Cystin dagegen in Disu.-fidform (-S-S) vorhanden ist, waren wir geneigt dem — SH Radikal im Cystein die wesentliche fungistatische Wirkung zuzuschreiben. Diese Annahme wurde durch folgende Untersuchungsergebnisse wahrscheinlich gemacht:

1. Zur Ausschaltung des -SH Radikals wurde die Bindung an Arsen angewendet. Als Arsenpräparat diente 3-amino-4-hydroxyphenyl-arsenoxyde (Arsenoxide) in der Handelsform Mapharside der Firma Parke, Davis & Co. Detroit. Wie nämlich aus den Untersuchungen von SIMON hervorgeht, erfolgt sofort nach Mischung von Cystein mit dem Arsenoxide eine chemische Bindung in der Art, dass ein Arsenatom zwei -SH Moleküle bindet. Bei den erwähnten Untersuchungen handelte es sich darum, das Arsen durch Bindung an -SH in seiner bakteriostatischen Wirkung zu hemmen und damit zu entgiften. Zu einer solchen Entgiftung ist, wie die Versuche des genannten Autors ergeben haben, die acht- bis sechzehnfache der theoretischen Cysteinmenge notwendig.

Im eigenen Fall war es dagegen nur wichtig alle freien -SH Gruppen mit Sicherheit auszuschalten. Es wurde deshalb nur eine teilweise Antagonisierung des Arsenoxide herbeigeführt, entsprechend der fünffachen theoretischen Cysteinmenge, also einem Verhältnis Cystein-Arsenoxide von 10 : 1, wo gewiß keine freie -SH Gruppe als Wirkungsfaktor in Frage kam. Wegen der toxischen Wirkung des noch verbliebenen nicht antagonisierten Arsenoxide mußte ein Pilzstamm von geringer Arsenempfindlichkeit verwendet werden. Nach einigen Vorversuchen wurde Aspergillus malignus Lindt gewählt. Bei allen anderen Stämmen außer Aspergillus malignus Lindt war der untenbeschriebene Effekt wegen der großen Arsenempfindlichkeit nicht zu beobachten.

Versuchsmethode: 0,6 gr Mapharsid wurden in 12 ccm sterilen destillierten Wasser gelöst und nach 20 Minuten Abstehen mit dem Nährmedium in einer Konzentration von 50 mgr% gemischt, also 1 ccm der angegebenen Lösung auf 100 ccm geschmolzenes Kulturmedium. Zum Herstellen der Cystein-Mapharsid-Röhrchen wurden 1 ccm der 5 %igen Mapharsidlösung mit 0,5 gr Cystein hydrochlorid gelöst und dann mit Nährmedium auf 100 ccm aufgefüllt. In die einzelnen Röhrchen wurden jeweils 10 ccm des betreffenden Mediums pipettiert. Die Versuchsreihe hatte dann folgendes Aussehen:

Röhrchen	1	2	3	4
	Nährmed. + Cystein 1 : 200	Nährmed. + Mapharsid 1 : 2000	Nährmed. + Cystein 1 : 200 + Mapharsid 1 : 2000	Nährmed. ohne Zusatz als Kontr.

Ergebnisse: Die Abb. 1 zeigt Aspergillus malignus Lindt nach 14 Tagen Bebrütung bei 35°. Im Röhrchen 3 ist trotz unvollständiger Antagonisierung des Arsens ein stärkeres Wachstum aufgetreten als in Röhrchen 1 mit Cystein allein. Freilich verhinderte das nichtgebundene Arsen das Auftreten eines so starken Wachstums wie im Kontrollröhrchen 4.

Daraus ergibt sich, daß nach Wegfall des Sulfhydril-Radikals die fungistatische Wirkung von Cystein ausgelöscht ist und somit der Sulfhydrilgruppe die wesentliche fungistatische Wirkung zuzuschreiben ist.

2. Um weitere Sicherheit zu gewinnen, daß das -SH Radikal das Wesen der fungistatischen Wirkung ausmacht, wurden Versuche mit Thioglykolsäure bei ph 5,5 angestellt. In der Thioglykolsäure ist ebenso wie im Cystein eine freie Sulfhydrilgruppe vorhanden. Diese Versuche zeigten in festem und flüssigen Czapek-Dox Medium bei einer Konzentration von 400 mgr % Thioglykolsäure die gleiche Wachstumshemmung wie mit Cystein.

3. Da Cystein im neutralen und alkalischen Bereich sehr leicht zu Cystin oxydiert wird, erprobten wir auch diese Oxydation zur Ausschaltung des -SH Radikals. Durch Zusatz einer 0,5 % Kalium-permanganatlösung zu dem auf ph 7 eingestellten Nährmedium war es möglich sowohl die Wirkung der Thioglykolsäure als auch des Cystein vollständig aufzuheben.

Besprechung der Ergebnisse: Die Ergebnisse der angeführten Versuchreihen zeigen deutlich, daß dem -SH Radikal sowohl im Cystein als in der Thioglykolsäure eine wachstumshemmende Wirkung auf Fadenpilze zukommt. Über die Art dieser Wachstumshemmung sollen hier keine Vermutungen angestellt werden. Ob das -SH Radikal ähnlich wie dies von Robbins für die OH Gruppe im Hydroxyprolin vermutet wird, ebenso in Fermentsysteme eingreift, müssen weitere Untersuchungen aufklären.

Die fungistatische Cystein — und Thioglykolsäure-Wirkung wurde durch Zusatz von nativem Menschenserum (5 ccm auf 100 ccm Nährlösung) vollkommen aufgehoben, ähnlich wie dies von Tager und Danowsky für die pilzwachstumshemmende Wirkung von Thiourea festgestellt wurde. Eine Aufhebung der fungistatischen Wirkung, ähnlich wie sie Iwanowic für die Salicylat-Bakteriostase mit Pantothen gefunden hat oder Snow in der Aufhebung der Sulfonamidwirkung auf Fadenpilze durch p-Aminobenzoesäure, konnten wit mit ähnlichen Stoffen nicht herbeiführen. Wir untersuchten β-Alanin, Natrium pantothenicum, Inositol und p-Aminobenzoesäure in Konzentrationen von 1 : 50.000 bis 1 : 1000.

Zusammenfassung

Es wird die Wirkung schwefelhaltiger Aminosäuren auf das Wachstum pathogener Dermatophyten in vitro untersucht. Dabei zeigt sich eine deutlich fungistatische Wirkung von l-Cystein hydrochlorid und Thioglykolsäure in Konzentrationen von 1 : 2000 aufwärts. Als Ursache für diese Wachstumshemmung wird das -SH Radikal angesehen, da dem Cystin, wo der Schwefel in Disulfdform vorliegt eine ähnliche Wirkung nicht zukommt. Durch verstärktes Wachstum von Aspergillus malignus Lindt nach Ausschaltung der -SH Gruppe mittels Bindung an Arsen, sowie Oxydation der Sulfhydril- zur Disulfidform wird diese Annahme wahrscheinlich gemacht.

Für die Erforschung der Dermatomycosen bei Mensch und Tier mögen diese Ergebnisse eine gewisse Bedeutung haben, zumal das Cystein in der Säugerhaut als Redoxsystem eine große Rolle spielt.

An dieser Stelle möchte ich Herrn Professor ROBBINS vom Botanischen Garten in New York für die Überlassung einiger Pilzstämme meinen ergebensten Dank aussprechen.

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Critical Survey of the Mycological Literature of the years 1939 to 1942

by

TIBOR BENEDEK, M. D.

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C. General biology, biochemistry and therapy

CASTELL and BRYANT (1939) investigated *the significance of color changes in dyes used for the detection of microbial action on fat.*

The mechanisms underlying color changes in fat globules in a medium containing any one of the three dyes studied in this paper are not always simple reactions of the same type. Therefore such color changes cannot be used as specific indications of any one type of reaction (i.e. hydrolysis of the fat). The lower fatty acids, either alone or mixed with fat, have the power of extracting these three dyes from an aqueous solution. If the dyes are in a colorless form in the aqueous solution, they are changed to their colored forms after being abstracted by the acids. Some substances other than fatty acids are able to extract the dye from the aqueous to fatty material. It is probable that most of the color changes observed in fat emulsion are due to reactions of this type. In the case of nile blue sulphate, besides extraction of the blue from the aqueous solution by fatty acids or other substances within the globules, similar color changes may be brought about by the reduction of the oxazone from a pink to a blue color. Fatty acids will not reduce the oxazone from pink to blue.

GRIGORAKI and DAVID (1939) investigated the *biochemical characteristics of endodermophyton indicum,* Castellani 1911. The investigation on different mediums (milk, gelatine, different carbohydrate solution with lithmus) have shown the following results: this fungus showed a less effective *casease,* but a very strong *trypsin* already at a minimal growth. Carbohydrates (glucose, mannose, galactose, saccharose, lactose, maltose, inulin, dextrin), and glycerol have been attacked only slightly. The biochemical characteristics of this fungus resembled that of *achorion violaceum* with which this fungus is closely related.

HOFFMAN, SCHWEITZER and DALBY (1940) made a *comparison of the fungistatic properties of enanthaldehyde and enanthic acids related to their possible anticarcinogenic effect.*

Since enanthaldehyde is only slightly soluble in water, dilutions in petroleum ether were made. The proper amounts of petroleum ether solution were pipetted into Petri dishes, and the dishes held at room temperature until the ether had evaporated. The buffer solution and media were then added. Petroleum ether, in quantities equivalent to the largest amount used in the dilution, was added to the control plates and allowed to evaporate before adding the buffer and media. No effect on mold growth was observed.

Enanthaldehyde has marked fungistatic properties over the pH range

2 to 8, but is not as powerful a fungistat as enanthic acid. The metabolism of aldehydes in rat-liver tissue has been studied (EDSON, 1936) and it was found that, although enanthaldehyde did not produce enanthic acid under anaerobic conditions, aerobically both substances were equally ketogenic. It has been shown recently that the tissues contain a distinct aldehyde mutase system (DIXON and LUTWAK-MANN, 1937). This evidence is suggestive that the first step in the metabolism of enanthaldehyde is its oxidation to enanthic acid, perhaps by the CANNIZZARO transformation. It is, therefore, at least possible that the anticarcinogenic effect of enanthaldehyde obtained by STRONG is due to enanthic acid produced by the tissues from enanthaldehyde. If, however, enanthaldehyde undergoes β-oxidation without going through the enanthic acid stage, the resulting shorter chain acids may also be anticarcinogenic, as suggested by the previously reported fungistatic properties of these compounds (HOFFMAN, et al, 1939).

PECK, MARTIN and HAUSER (1940) investigated the *polysaccharides of Blastomyces dermatitidis.*

In diseases caused by pathogenic yeast-like fungi, the polysaccharides of these organisms appear to play an important part. Animals immunized with the whole cells of some fungi, such as *Monilia albicans,* develop circulating antibodies capable of precipitating the isolated polysaccharides. In some instances, however, hypersensitivity to the polysaccharides may develop in the absence of demonstrable precipitins. Such hypersensitivity is probably of importance in certain cases of chronic infection. This is especially true in some patients with American blastomycosis (GILCHRIST'S disease). In view of this fact, it is of interest to report some data on the preparation, properties and biologic activities of certain polysaccharides which were obtained during chemical studies of *Blastomyces dermatitidis.*

Isolation of polysaccharides. To obtain preparation I of polysaccharide fresh cells of *Blastomyces dermatitidis* were grown at 37° C. for seven days on a solid medium. The growth was carefully scraped from the medium, suspended in M/8 saline solution containing 1 per cent of phenol, and allowed to stand at room temperature for four days. The cells were then removed by centrifugation, and to the supernate was added 10 per cent of sodium acetate. After making the solution slightly acid to litmus with acetic acid, it was treated with 1½ volumes of 95 per cent alcohol and left over night in the cold. The resulting precipitate, collected by centrifuging, was redissolved in water and the proteins removed by shaking with chloroform and amyl alcohol according to the method of Sevag. Ten per cent of sodium acetate was added to the crude protein-free polysaccharide solution, and after acidifying to litmus with acetic acid, 1½ volumes of 95 per cent alcohol were added. The polysaccharide was collected by centrifuging. After this process had been repeated eight times, the substance was precipitated again without the addition of sodium acetate. The resulting precipitate was redissolved in distilled water and poured into ice-cold acetone with constant stirring. The flocculant precipitate was then filtered on hardened paper, washed with acetone and ether, and dried in vacuo over sulfuric acid. The yield from 10 grams (dry weight) of cells was 0.1 gram of a faintly buff-colored hygroscopic powder.

Preparation II of polysaccharide was isolated from the residual aqueous solution left after removal of lipids from the alcohol-ether extracts of fresh cells of *Blastomyces dermatitidis.* After precipitating it from the concentrated solution by the addition of 2 volumes of 95 per cent alcohol, the crude preparation was redissolved, chilled and treated with 10 per cent of trichloracetic acid to remove proteins. The polysaccharide was further purified as described above for deproteinized preparation I and was finally obtained as a faintly buffcolored hygroscopic powder. Analysis: N, 1.0; P, 0.3; C. 38.0; H, 6.9; Ash (as Na) 11.1. C and H were calculated on ash-free basis. The yield was 1.2 grams from extracts of 170 grams (dry weight) of cells.

One gram of preparation I was fractionally precipitated with alcohol. The crude polysaccharide was dissolved in 100 ml. of 10 per cent aqueous sodium acetate, acidified with one drop of 50 per cent acetic acid, and treated with 80 ml. of alcohol. After standing over night in the cold, the precipitate designated fraction C—1, was collected by centrifuging. The clear supernate, volume 180 ml., was treated with 70 ml. of alcohol, causing the appearance of further precipitate, fraction C—2, which was collected as before. By treating the supernate from fraction C—2 with 300 ml. of alcohol, nearly all of the remaining polysaccharide material was precipitated and fraction C—3 isolated as above. The three fractions of preparation II were further purified by the method described above for deproteinized preparations.

Chemical analysis of *Blastomyces dermatitidis* indicates that this fungus contains at least two distinct polysaccharides. Minute quantities of these polysaccharides fix complement with sera prepared by immunizing rabbits with whole *Blastomyces dermatitidis.* Patients with American blastomycosis who are in the allergic state of the disease respond to intracutaneous injection of the polysaccharide with a swelling which reaches its maximum within 24 hours. Experience with one patient has shown that intravenous injection of a solution of this polysaccharide not only results in a decrease of carbohydrate sensitivity but also abolishes the sterile abscess-formation produced in an allergic patient by injection of heat-killed whole organisms.

Adelia Mc Crea (1940) undertook a critical review of *fungicidal testing: A comparison of methods.* She commented upon three methods for making fungicidal tests: those advocated by Reddish, by Klarmann, and by Taft, suggesting a method which shows more clearly the power of a vigorous fungus to survive adverse conditions. Details of the method are outlined briefly below.

Trichophyton interdigitale Priestley and *Epidermophyton rubrum* Cast., two other dermatophytes are usually tested, *Achorion gypseum* Bodin and *Microsporum lanosum* Sab. Frequently *Aspergillus niger* Tieghem is included when information is desired as to the effect of the test substance on laboratory weeds.

I. Spore Suspension. Here is where the crux of the test really lies. A poorly prepared, deteriorated suspension is much easier to kill than one which contains the spores and mycelial elements of a normally vigorous, sporulating fungus.

In growing the cultures of skin fungi for test purposes, test tubes 18 by 140 mm. are used, with 8 c.c. of Sabouraud's glucose agar, slanted to leave a butt of about onehalf inch, and planted while fresh so that growth will cover the entire surface. (Equivalent surface in flask cultures would, of course, do as well). When mature growth has occurred, as described above, suspension is prepared by washing the surface with sterile, distilled water. For such species as *Monilia* and related forms, 10 c.c. per tube are used; for such cultures as *Aspergillus* and *Penicillium,* 12 c.c. per tube; for dermatophytic fungi, 8 c.c. per tube. The underlying medium should never be broken, but spores may sometimes need to be dislodged by gentle strokes of a bent wire or a loop.

Spores and mycelial fragments are prone to "clump" in groups, hence the suspension should be strained through sterile cloth — double layer, if gauze; single, if mull is used. This gives a homogeneous suspension with microspores (conidia), macrospores ("fuseaux"), and mycelial cells throughout the liquid. Because of these multiple elements in a fungous suspension, a counting chamber of value has not been found, and rely upon the growth obtained from a comparable area of a thrifty culture at a definite age to assure a comparable suspension; it is believed this to be as nearly exact as one can reach in handling living materials. Control tubes are planted at the beginning and end of each dilution tested.

II. Test Substance. Dilution Series. There is no magic formula for a dilution series. The usual Food and Drug Administration method is readily applicable, except that planting should be made upon agar medium instead of liquid. In most cases, a preliminary, wide dilution series will set the limit for the final series of dilutions.

III. Exposure Intervals. One, five, ten minutes, and one hour exposure intervals are used. When fungistatic effect is to be studied, daily transplants are made for any desired period. Customarily three weeks are allowed.

IV. Incubation Period. For short life-cycle fungi, as *Monilia, Cryptococcus,* etc., final records may be taken seventy-two hours after planting.

For longer life-cycle species, as the dermatophytes, the Aspergilli, the Penicillia, etc. records should not be considered complete until at least one week after testing. Germination often may be delayed a few days, with a good growth appearing later.

For practical purpose, no other method yet devised is as satisfactory as this in vitro (test tube) method to give comparative criteria on liquid fungicidal agents. Its outstanding advantages are:

1. It uses only thrifty, mature cultures with their spores well developed as they are found in nature.
2. After the suspension has been properly prepared, this method is closely comparable to the well-known official method of testing germicides. (Federal Drug Administration, with or without the Shippen modification). Adequate controls aid in correct interpretation of results.
3. It gives ample scope for individual choice of dilution series and time intervals.
4. Its results can be check-tested without much delay, provided a sufficient number of cultures was prepared in the original planting. In laboratories where much testing is carried on, cultures may be planted routinely at two- to three-week intervals so that they are available at all times in a suitable stage of development.

Bacteriologists, chemists, and general technicians should realize the necessity to consider the mycologic nature of species which they use in any fungicidal test. True data and fair comparisons are not possible unless this is done.

GEORGI and ETTINGER (1940) investigated the *utilization of carbohydrates and sugar by the Rhizobia.*

Growth and fermentation characteristics of six species of *Rhizobia*, several strains of Alcaligenes radiobacter and one of Azotobacter chroococcum on a number of sugars and salts of sugar acids are reported. In general, mono- and disaccharides were more readily utilized than tri- or polysaccharides and the sugar acids. The fermentation of the former resulted in the production of an acid reaction while the tri- and polysaccharides and sugar acids made the media alkaline. Alcaligenes radiobacter and Azotobacter chroococcum were capable of growing abundantly on practically all organic carbon sources. Methods of preparation and sterilization of the carbohydrates yielded no apparent differences in ease of availability or fermentation character, with any of the organisms or carbon sources investigated, except perhaps, amygdalin. Efficient and inefficient strains of *Rhizobia* cannot be differentiated physiologically on the basis of fermentation reactions. Sodium and calcium salts of sugar acids, with the exception of calcium 5-keto gluconate, are capable of supporting the growth of the microorganisms studied. Alcaligenes radiobacter may be distinguished from the *Rhizobia* by a variety of growth and fermentation reactions, notably on sodium 1-mannonate and calcium 5-keto gluconate. Changes in reaction and reversions in pH, produced by *Rhizobia,* Alcaligenes and Azotobacter on various media, are discussed.

Castell and Garrard (1940—'41) investigated the *action of microorganisms on fat.*

Observations (1940) of uninoculated globules of triglycerides and fatty acids and of butter fat in an agar medium showed the following results:

With the object of discovering the significance of the "opaqueness" surrounding so called lipolytic colonies on fat-emulsion agar plates, detailed observations have been made on uninoculated globules of butterfat, pure triglycerides, fatty acids, and mixtures of fats and fatty acids. The following observations have been made: At room temperature, sooner or later, fat crystals form in all butterfat globules. The rapidity with which crystals form and their general conformation and texture are capable of considerable variation. This depende upon the previous history of the butterfat, temperature and the physical and chemical characteristics of the agar medium in which the fat is suspended. Sodium chloride, among other substances, brings about rapid changes in the form of the globules and the texture of the crystals. The crystals of pure fats suspended in agar are quite unlike the crystals in globules of butterfat. When these same fats are suspended in some form of oily matrix this difference disappears. The chief difference between pure fats and fatty acids, when suspended in oil, is that there is a tendency for the fatty acids to crystalize at the fat-agar interface, while the fats do not. The lower, solid fatty acids often protrude out into the agar medium. Copper sulphate and Nile blue sulphate, as well as a large number of other dyes have been used to differentiate fatty acids from fats. It appears probable that the phenomenon of opaqueness in the fat globules surrounding lipolytic colonies in fat-emulsion agar plates is owing, at least in part, to the rapid formation of fat crystals, as well as to the formation of fatty acids.

Investigations (1941-a) on oxidation and hydrolysis of triolein by pure cultures of bacteria revealed the following results:

The hydrolytic activity of 40 pure cultures of bacteria on triolein, as indicated by increased titratable acidity, has been recorded. Schiff and Kreis tests have been made on similar samples of triolein acted upon by pure cultures of bacteria, and the results of these tests are compared with the "oxidase reaction" of the individual cultures. The results indicate that most of the Gram-negative lipolytic organisms also oxidize the fat, and that appears to be a definite relation between the "oxidase reaction" of a bacterial colony and the ability of the organisms to produce oxidative rancidity.

Observations (1914-b) on the changes produced in globules of triolein by pure cultures of bacteria permitted the following deductions:

A series of observations have been made on the action of 60 cultures of bacteria on globules of triolein in oil emulsion agar media. Lipolytic activity as indicated by the color reactions of Nile blue sulphate and methylene blue and the blue soap formation with copper sulphate has been shown to coincide with that of the same organisms as measured by the titratable acidity they produced in larger samples of the oil. Other Eh indicators have been shown to color the globules around lipolytic colonies. Other changes in the color and texture of the globules have been shown to coincide with the oxidative activity of the bacteria as measured by KREIS and SCHIFF tests on larger samples as well as by the oxidase reaction of the bacterial colony. Preliminary hydrolysis appears to hasten and intensify the oxidative reactions as indicated by the globules. Other color reactions in the globules and the formation of various types of crystals have been observed and their significance discussed.

AREA LEAO (1941) in a detailed study on the pathogenesis of ringworms, emphasized the peculiar affinity of these parasites to the dead layer of the skin and its appendages, adding that there is not a matter of tropism, as observed in other diseases. He studied the resemblance of the infection through dermatophytes to other diseases produced by virus and located on the skin. References are made to the infection by cutaneous and hematagenous ways, stress being laid upon the inadaptability of the parasite to the internal medium. He studied the metabolic products of these parasites — the tricophytin — and the advantage of its use for the diagnosis of tineae. At last he studied the allergy phenomena in ringworms and the role they play in the evolution of the disease. References are made to the relationship between immunity and allergy in these forms of parasitism; classification and description are given of the more frequent allergens as well as of the rare clinical forms described up to now.

CASTELL (1941) made experiments with *p-aminodimethylaniline monohydrochloride as an indicator of microbial action on fats.*

Flooding p-aminodimethylaniline monohydrochloride on fat emulsion agar inoculated with certain types of microorganisms frequently results in marked color changes in the fat globules. It is shown that the colors result from the increased solubility in fat and fatty acids of this dye as it becomes oxidized. Some of the acids oxidize the dye on contact and therefore color very quickly; fats become colored only when some other agent oxidizes the dye. The characteristic color reactions with certain fats and fatty acids are described for various degrees of oxidation of the dye; this suggests the explanation for the colors observed in the inoculated globules flooded with this dye.

Color reactions in fat globules inoculated with pure cultures of bacteria and flooded with p-aminodimethylaniline monohydrochloride and compared with the color reactions of the same organisms with nile blue sulphate as an indicator

Organism	Red	Blue	Amber Colored	Blue with Nile blue sulphate
Bacillus subtilis	—	—	—	—
Bacillus mycoides	—	+?	—	+?
Bacillus panis	—	—	—	—
Bacillus cereus	—	+	—	+
Bacillus graveolens	+	—	—	—
Bacillus mesentericus	++	—	—	—
Staphylococcus aureus	—	—	++	+
Staphylococcus citreus	—	—	+	—
Streptococcus lactis	—	—	—	—
Micrococcus conglomeratus . . .	+	—	—	—
Diplococcus capsulatus	—	—	—	—
Sarcina lutea	—	—	+?	—
Sarcina aurantiaca	—	—	—	—

Organism	Red	Blue	Amber Colored	Blue with Nile blue sulphate
Erwinia carotovora	++	—	—	—
Rhizobium radicicola	++	—	—	—
Phytomonas campestris	+	+	—	+
Achromobacter putrefaciens . . .	++	—	—	—
Achromabacter lipolyticum . . .	+	+	+	+
Pseudomonas fluorescens	++	+	+	+
Pseudomonas aeruginosa	++	+	—	+
Pseudomonas fragi	++	+	—	+
Pseudomonas mucidolens	++	+	—	+
Alcaligenes viscosus	—	—	+	—
Alcaligenes lipolyticum	+	+	—	+
Alcaligenes faecalis	++	—	—	—
Brucella abortus	++	—	—	—
Eberthella typhosa	—	—	—	—
Aerobacter aerogenes	—	—	—	—
Aerobacter cloaceae	—	—	—	—
Salmonella (9 species)	—	—	—	—
Proteus vulgaris	—	—	—	—
Escherichia coli	—	—	—	—
strong reaction ++ . . .				
weak reaction + . . .				
negative reaction — . . .				

Robbins, Mackinnon and Ma (1942) investigated the *vitamin deficiencies of Trichophyton discoides.*

T. discoides is parasitic on calves and produces an infection of man which presents the same clinical characteristics. *T. album* is very similar to *T. discoides* in its pathogenic effects and is distinguished from the latter organism by differences in the appearance of colonies upon Sabouraud's medium. However, *T. discoides* closely resembles *T. album* when grown on Sabouraud's dextrose medium to which thiamine had been added and an investigation of *T. album* demonstrated that it synthesized thiamine. This suggested that *T. discoides* may be a mutant of *T. album,* a mutation which involved the loss of the ability to synthesize thiamine. These observations emphasize also the importance of the vitamin content of a medium in determining the diagnostic characteristics of an organism which is identified largely by its colonies.

T. discoides is the only filamentous fungus known to the authors to have a complete inositol-deficiency.

The complete inositol-deficiency shown by *T. discoides* suggests that this organism might be used for detecting and perhaps estimating inositol. However, before complete dependence can be placed on its use for the bio-assay of i-inositol further studies on specificity similar to those made on yeast should be made with this organism. The relatively slow growth of *T. discoides* and the need for an incubation temperature of 35° to 38° C. are disadvantages. The present observations with this organism suggest that it may be useful in detecting the presence of inositol. For example, by bio-assay with *T. discoides,* it appears that agar contains little or no inositol while gelatin, casein hydrolysate, and albumen hydrolysate contain appreciable amounts

T. discoides suffers from complete deficiencies for pyridoxine, i-inositol, and molecular thiamine. In addition there are partial deficiencies for unidentified substances. The unidentified substances were present in peptone, casein hydrolysate, hydrolyzed egg albumen, malt extract, gelatin, and a filtrate (DR fraction) prepared from white potatoes. No evidence was obtained that the partial deficiencies could be satisfied by biotin, lactoflavin, folic acid, pimelic acid, para-aminobenzoic acid, pantothenic acid, hypoxanthine, guanine, nicotinamide, 2-methyl-1,4-naphtho-quinone diacetate, seven pyrimidine and purine bases, and 43 amino acids. Ca phytate and a phosphatide containing inositol were less effective than i-inositol. The presence of appreciable quantities of inositol in gelatin, casein hydrolysate, and albumen hydrolysate was indicated by the growth of *T. discoides*. Unhydrolyzed agar appeared to contain little or no inositol. Maximum growth was obtained in a 4 weeks period in a medium containing between 0.1 and 0.5 mg of inositol, and between 1 and 10 μ moles of pyridoxine and thiamine.

ROBBINS and MA (1942) investigated the biologic interrelationship of *pimelic acid, biotin and certain fungi.*

The following organisms were used in one series of experiments: *Ceratostomella ips* #255, *C. ips* #438, *C. microspora, C. montium, C. obscura, C. penicillata, C. pini, C. radicicola, Grosmannia serpens, Fusarium avenaceum, Neurospora sitophila* 56.2 and *N. tetraspora* S_1. None of these fungi makes more than slight growth on a mineral-dextrose medium containing asparagine and purified agar unless biotin is present. The addition of 0.05 μ g of biotin to a tube containing 8 ml of the basal medium permits luxuriant growth.

Negative results were obtained when the 0.05 μ g of biotin was replaced with 0.05 μ g of pimelic acid. No benefit was observed when the quantity of pimelic acid was increased to 0.1 μ g per tube containing 8 ml of medium.

Sulfur is furnished in the basal medium as $MgSO_4$.

Ashbya (Nematospora) *gossypii*, the organism used by KÖGL as a means of bioassay in the original isolation of biotin, was also tested. Negative results were obtained when 1 μ g or 100 μ g of pimelic acid were added in place of biotin to 8 ml of a basal medium and when biotin was replaced with 1 μ g or 100 μ g of pimelic acid together with 1 mg of l-cystine. The results were negative also when the medium containing pimelic acid or pimelic acid and l-cystine was further supplemented with 1.5 mg of casein hydrolysate per tube.

It appears that the thirteen fungi used are not able to synthesize biotin from pimelic acid, or from pimelic acid and l-cystine, under the experimental conditions. This should not be interpreted to mean that other organisms can not construct biotin from pimelic acid and l-cystine, nor that pimelic acid is not a precursor of biotin. The relation of microorganisms to thiamine and its thiazole and pyrimidine intermediates have demonstrated that some organisms have no synthetic power for thiamine and require it in molecular form; others have incomplete sythetic power and can construct the vitamin if furnished the proper intermediates, but not otherwise; while still others are able to make thiamine from the minerals and sugar in a basal medium. A somewhat similar situation may exist with regard to biotin. If it does, the fungi which have been used appear to require biotin as such.

ROBBINS (1942) tested a *pyrimidine analog of thiamine and the growth of fungi.*

The compound pyrimidine was tested with three fungi, *Phycomyces Blakesleeanus, Pythiomorpha gonapodyides* and *Phytophthora cinnamomi*, each of which has a different kind of thiamine deficiency.

Each organism was grown in 25 ml quantities of a solution containing per liter 50 g dextrose, 1.5 g KH_2PO_4, 0.5 g $MgSO_4$, 2.0 g asparagine and the following mineral supplements in p. p. m.: 0.005 B, 0.02 Cu, 0.1 Fe, 0.01 Ga, 0.01 Mn, 0.01 Mo and 0.09 Zn. To this solution various amounts of the analog, of thiamine, of thiazole or of pyrimidine were added. The solutions were sterilized for 20 minutes at 12 pounds pressure and each treatment was carried out in triplicate. The temperature of incubation was 20° C. *Phycomyces* was grown for 8 days, *Phytophthora* for 11 days and *Pythiomorpha* for 11 days in the first experiment, and 15 days in the second experiment. At the end of the period of growth the mycelium was removed from each flask, washed with distilled water, dried at 100° C. and weighed.

The pyrimidine analog was neither beneficial nor detrimental to *Phycomyces* even when 1000.0 μ moles were added per flask. The addition of pyrimidine to the basal solution containing 2.0 μ moles of the analog did not improve growth. These results are to be expected since all previous evidence shows that both pyrimidine and thiazole must be available for growth of *Phycomyces* to occur. Addition of thiazole to 2.0 μ moles of the analog had little effect as may be noted by comparing the growth obtained with the combination and in the solution containing 2.0 μ moles of thiamine. However, when larger quantities of the analog (10.0, 100.0 or 1000.0 μ moles) were used in the presence of 100 μ moles of thiazole considerable growth developed. The dry weight with 100.0 μ moles of the analog in the presence of 100.0 μ moles of thiazole was about the same as that found with 0.1 μ moles of the analog in the presence of thiazole produced more dry weight than 1.0 μ mole of pyrimidine and 100.0 μ moles of thiazole. The analog was ineffective as a substitute for thiamine for *Phycomyces* but it was about 1/1000 as effective as thiamine as a source of pyrimidine.

Pythiomorpha grows if furnished with the pyrimidine half of the thiamine molecule; it is able to synthesize the thiazole portion from the sugar, minerals and asparagine in the basal medium. The analog was not injurious to *Pythiomorpha*, in fact 10.0 μ moles definitely improved its growth and 100.0 or 1000.0 μ moles were still more favorable. However, less growth was obtained with 100.0 μ moles of the analog than with 0.5 μ mole of thiamine. In the first experiment somewhat more growth occurred with 1000.0 μ moles of the analog than with 2.0 μ moles of thiamine. In the second experiment 1000.0 μ moles gave about the same yield as 2.0 μ moles of thiamine and somewhat more than 0.5 μ mole of thiamine or of pyrimidine. As a source of pyrimidine for *Pythiomorpha* the analog appeared to be about 1/500 as effective as thiamine.

Phytophthora will not grow unless supplied with molecular thiamine. The analog, even in amounts of 1000.0 μ moles per flask was ineffective with this organism.

The response of the three fungi leads to the following conclusions: The pyrimidine analog of thiamine does not replace thiamine in the physiology of these organisms. Its effectiveness as a source of pyrimidine for *Phycomyces* or *Pythiomorpha* is of the order of 1/500 or 1/1000 that of thiamine. The action of the analog as a source of pyrimidine might be ascribed to the presence of traces of pyrimidine as an impurity or more probably to a slight dissociation of the compound into its constituents or to decomposition in sterilization. Neither *Phycomyces* nor *Pythiomorpha* appears able to split the compound and obtain pyrimidine from it. In this respect the pyrimidine analog differs from the pyridine analog studied earlier; the latter compound was about as effective a source of pyrimidine as thiamine.

MACKINNON (1942) investigated the *effect of Actinomyces albus and of thiamin on the growth of Trichophyton discoides*.

The fungus *Trichophyton discoides* SABOURAUD is a common cause of tinea in calves in Uruguay. It is also a frequent cause of tinea of the scalp, the beard, and the glabrous skin of man. These infections are usually very inflammatory. On SABOURAUD's media, *T. discoides* grows slowly and produces a flat, smooth, and usually glabrous giant colony; some strains produce a central umbo and short velvet; the colony has a brownish neutral color. SABOURAUD did not describe conidia; but in 1934 LEBASQUE observed aleurospora and closterospora in cultures on different cereals and on horse dung. CAZALBOU describes phases in the cultures of *T. discoides*; where *T. discoides* is mentioned in this paper the so-called normal phase is to be understood.

During study of the strain 688, a culture on glucose-peptone agar was accidentally contaminated by an Actinomyces of the *Actinomyces albus* group. *A. albus* (strain 693) began to grow at a distance of four cm from a colony of *T. discoides*. After 15 days the sector of the colony of *T. discoides* nearest to the colony of *A. albus* became folded and velvety while the other sectors remained smooth and glabrous. Folding of the colony was due to an increase of the growth. The fact was confirmed many times. If the agar between the two colonies was cut, the Actinomyces had no further influence over the growth of the Trichophyton.

The same results were obtained with another strain of the *Actinomyces albus* group, isolated from the soil (strain 800). A successful attempt was made also to increase the growth of another strain of *T. discoides* (strain 461).

Strains of Actinomyces, of the *Actinomyces albus* group, have a stimulating influence over the growth of *Trichophyton discoides* Sabouraud, when the latter is grown on glucose bacto-peptone agar. The giant colonies, usually flat, smooth, and glabrous, become folded and velvety. The addition of thiamin to the glucose bacto-peptone agar has the same effect as A. albus on the growth of *T. discoides*. An agar medium lacking thiamin to which cultures of *A. albus* on glucose Czapek medium have been added permits *Phycomyces Blakesleeanus* to produce long sporangiophores as well as if thiamin had been added. Filtered cultures of *Actinomyces albus* in liquid Czapek medium have a similar but less marked effect.

K. OVCHAROV made investigations on the *ferment of pathogenic fungi* causing the splitting off of urea from protein. Urea and similar compounds play an important role in the nitrogen metabolism of fungi. Also the phytopathogenic fungi produce urea and to a lesser extent thio-urea. In nutrient media with proteins some of the pathogenic fungi shows an increased urea-production. The author describes his experiments for the quantitative determination of the influence of ferments split off from proteins. It concerned the effect of ferments getting into the nutrient media during the growth of the fungi, and that, recovered from the mycelium (endoferment). The principle of the method is based upon the determination of the amount of urea split off in the process. After incorporation of urease and phosphate buffer solution (for 3 hours in thermostate at 33° C.) the developing ammonia was distilled in vacuum at 40—50° C. and the amount determined. The strength of the "Deurase" depends on the contents of nitrogen compounds in the medium and in presence of proteins on its kind. The larger part of Deurease is contained in the mycelium, small amounts are excreted into the nutrient medium.

ASTA V. MALLINCKRODT HAUPT investigated *the surface and depth growth of microorganisms in culture.* In comprehensive series with different fungi and bacteria she examined the influence of proteins, carbohydrates, vitamins, as well as the actual reaction of the nutrient media as to the changes of surface and depth growth of the colonies. It was assumed that surface growth represents a more intensive demand for oxygen and that depth growth reveals a more anacrobic respiration. The different fungi and bacteria demonstrated quite different attitude. The source of nitrogen appeared to be of decisive importance. Among the amino-acids, asparagin caused a pronounced surface growth, while cystin and leucin led to a marked depth growth. Carbohydrate seemed to be of less importance. It was, however, surprising that, for instance, B. coli-strains showed a heavy surface growth with scum formation only in cultures in which no fermentation of the incorporated carbohydrates took place. Among the vitamins vitamin B revealed the strongest influence causing a rather pronounced effect on surface growth and scum formation. Other vitamins did not reveal a uniform effect. No relations could be established between the direction of growth and the actual reaction. It was, however, conspicuous that relationship exists between the direction of growth (whether surface or depth), the type of respiration, the quality of the amino-acids present. Furthermore the influence of certain vitamins became conspicuous.

W. F. LEVER tried a new fungicidal preparation: *iodocholeate.* GOEDRICH has discovered a new iodine compound, iodocholeate, which is an iodine-choleate adsorption compound. Because the iodine in iodocholeate is absorbed to the bile salt, it is less volatile and combines less readily with protein. These two properties explain the advantages which iodocholeate has over tincture of iodine: 1. It is less irritating, as had been shown by patch tests. The ointment containing 2.5 per cent iodocholeate led to an erythematous reaction in 8 per cent of the cases and in no case to a vesicular reaction; the ointment containing 2.5 per cent tincture of iodine led to a reaction 38 per cent (erythematous in 32 per cent and vesicular in 6 per cent). The 1 per cent iodocholeate solution led to an erythematous reaction in 6 per cent and in no case to a vesicular reaction; the 1 per cent tincture of iodine led to a reaction in 22 per cent (erythematous in 20 per cent and vesicular in 2 per cent). 2. Iodocholeate has greater fungicidal power than tincture of iodine in the presence of protein. A lower concentration of iodocholeate than of tincture of iodine is required in Sabouraud's medium to prevent the growth of fungi. As to human skin and its exsudates contain protein one might expect a higher fungicidal action here also. 3. Iodocholeate is active over a longer period than tincture of iodine. The fungicidal power of SABOURAUD's medium containing tincture of iodine decreases in two weeks, while that of SABOURAUD's medium containing iodocholeate remains the same. Either in solution or incorporated in an ointment iodocholeate has proved itself valuable in the treatment of fungous diseases of the skin.

SAMUEL M. PECK, H. ROSENFELD, W. LEIFER and W. BIERMAN investigated the *role of sweat as a fungicide,* with special reference to the use of conctituents of sweat in the therapy of fungous infections. The comprehensive experimental and therapeutical investigation led to the following conclusions. Thermal sweat may have fungistatic and fungicidal properaties at a pH below 7. Concentrated sweat is fungistatic even when alkalinized. The fungicidal properties of sweat are due to its content of acetic, propionic, caproic, caprylic, lactic and ascorbic acid. These substances must be present in the proper concentration to exert a fungistatic or fungicidal effect. There seems to be a relation between the localization of fungous infection and the distribution of sweat on the surface of the body. Areas which are exposed to the greatest concentration of sweat seem to have less tendency to fungous infection. Sebum as exemplified by the contents of a sebaceous cyst, could not be shown to be fungicidal or fungistatic. Topical applications of ingredients of sweat, such as mixtures of lactic acid, propionic acid, butyric acid and ascorbic acid in proper concentration, have proved valuable in the treatment of fungous infections.

L. Grigoraki and Roger David investigated the *biochemical characteristics of Microsporum canis* (Bodin, 1897) Grigoraki and Giart, emend. 1928. Inoculation of the fungus in milk revealed the presence of a rather strong casease, inoculation in gelatine showed the effect of a strong trypsin. Investigations with different carbohydrates and glycerin with litmus as indicator demonstrated fermentative characteristics different from that of *Trichophyton crateriforme* and *Achorion violaceum*. The biochemical characteristics may be used in differentiation of dermatophytes.

Grigoraki, L., and Roger David investigated the *biochemical characteristics of Trichophyton lacticolor* (Sabouraud, 1910). In contradistinction with other fungous species, *Tr. lacticolor* revealed a rather active casease, active trypsin and a weak but conspicuous action on different carbohydrates and glycerol. Indol formation was uncertain. Furthermore they found that *Tr. asteroides* (Sabouraud, 1909) shows a mildly active casease and trypsin, and characteristic discoloration of litmus solution in the presence of diverse carbohydrates and glycerol.

E. Janisch examined the *optimal temperature for Aspergillus niger*. The optimum is characterized not alone by the quickest development and the most rapid growth. It is rather characterized by the spore formation, the duration of development from spore to spore, the growth of mycelium and the size of the crop. For all these factors, 31° C. was found as optimal. This temperature, rather independent from any other secondary or tertiary factors, must be regarded as species . . . or race-specific.

Razi Maner attempted the *serological differentiation of anaerobic Leptotricheae*. Rabbits injected intravenously with pure cultures of anaerob Leptothrix strains yielded sera showing agglutination not only with the homologous strain but with other strains of leptothrix and Leptothrix-like bacteria. In human serum one could establish in a few cases the presence of agglutinins for Leptothrix.

Meltzer observed in an asylum for weak-minded children among two hundred 27 cases of microsporosis (among them one girl) and treated them with Thallium according to the recommendation of Buschke. The untoward effects were rather insignificant, the hair fall was prompt and under subsequent topical treatment twenty cases were cured, seven "improved". One 16 year old boy who, however, did not show any sign of puberty yet, died suddenly. He was an epileptic.

A. Dosa recalling the *effect of sodium boracicum* ($Na_2B_2O_4$) against budding and filamentous fungi carried out in vitro experiments and found that the effect of Borax ($Na_2B_4O_7$) is not an alkali effect but a specific borax-effect. Further practical experiments demonstrated that borax in aquesous solution or as a salve is very efficient against superficial trichophytosis, microporum and favus infection of the glabrous (1 to 5 % and hairy skin (10—15 %).

St. Ballagi summed up his experiences based on an activity of three decades in the clinic for skin diseases of the University of Budapest concerning the *therapy of dermatomycoses*. There are three important points: (1) the vaccine, (2) desinfectant salves and lotions, (3) the epilation. The success of the vaccine therapy depends upon the following requirements: the vaccine must be specific; it must be prepared from virulent and not from degenerated fungous strains; during the preparation of the vaccine the efficient agents must not be deteriorated by the technic used; its dosage must be exact and the best way is the intracutaneous administration. The success of the treatment is decided by the allergic reaction: in case of positive reaction the vacine-therapy is certainly reliable. The vaccine-therapy can efficiently be supported by desinfecting salves and lotions: such as a 3—5 % salicyl-boric-acid salve, a 3—5 % salicyl-diachylon ointment, or a 10 % calcium hypochlorosum salve can be recommended; as to the lotions: 5 % tincture of iodine, 1 % chrysarobin or 10 % cadogel tincture in benzol, 1 % aqueous picric acid solution, Castellani's resorcinol-fuchsin-aceton-tincture; 1 % acid cinnamoneum 2 % alcohol tincture in chloroform are useful.

During epidemics of microsporosis, trichophytosis and favus, the epilation is the most important. The following principles must be observed. Up to the tenth year of age use of thallium alone is recommended (per kg body weight not more than 7 mgm, total use never higher than 250 mgm). For children of ten to fourteen years of age, a combined Rontgen-thallium epilation is considered the best (half of the x-ray epilation dose plus 4.5 mgm. Thallium per kg body weight). Children over fourteen years of age should be epilated by x-ray alone.

Vaccine-therapy is particularly recommended for erosio interdigitalis, parasitic dyshidrosis, oidiomycosis, trichophytosis and epidermophytosis of the palms and soles, eczematous patches caused by common molds. Topical application of desinfectants is important in primary onychomycoses. Before application a softening of the nail plates with a 20 % salicylic salve is recommended. Topical treatment is indicated in superficial trichophytosis, microsporosis and favus corporis. The deep seated forms of dermatomycoses (sycosis parasitaria, Kerion Celsi) can be cured with vaccine-therapy alone, or in connection with hot dressings of calc. hypochloros. solution. In cases of "ids" the emphasis is laid on vaccine-therapy.

H. SHARLIT recommended *iodine therapy in fungous infections.* The choice is a iodine compound which easily decomposes, giving off little by little iodine. Tetraiodmethenamin in collodion was efficient in foot mycoses. This compound is a direct addition product of iodine and methenamine, approximately 80 % iodine. Clinical experience with this iodide in a collodion vehicle for use on fissuring lesions of tinea of the feet was highly satisfactory.

T. OZZANO reported on the results obtained by *x-ray epilation* carried out in 300 children suffering form tinea of the scalp. The author found the x-rays is a powerful means for the treatment of the fungous infection. He describes his own special technique according to which he divides the scalp *into nine fields* in modification of the method of KIENBOCK-ADAMSON, giving 350r per field at 110 Kv max, 5 MA., of non-filtered rays. This new technic, the author believes, makes it possible to irradiate the scalp more rationally and with greater security against the danger of radiodermatitis.

BURLINGAME and REDDISH (1939) worked out *laboratory methods for testing fungicides used in the treatment of Epidermophytosis.*

The following fungi were used as test organisms: *Epidermophyton interdigitale, Trichophyton purpureum, Trichophyton gypseum, Epidermophyton inguinale,* and *Trichophyton rosaceum.* The laboratory method for testing for fungicidal activity employed simulates practical conditions more closely than those previously described and suggested for this purpose. This method is as follows:

Each organism is streaked over the entire surface of SABOURAUD's agar (20 c.c.) in a 9 cm. PETRI dish, using a dry sterile cotton swab inoculated with cultures of these organisms which had been grown on SABOURAUD's agar for five days at room temperature. These plates are incubated at room temperature for five days. The agar cultures are then cut into 1 cm squares, or into disks by means of a cork borer, 1 cm in diameter. The fungicide to be tested is poured over the surface of the cultures so as to entirely flood the plates, which requires 15 c.c. or more for each plate. After five, fifteen, and thirty minutes, one of the squares or disks of culture and agar from each plate is removed and placed into 10 c.c. of sterile broth. The excess fungicide is washed out of the matted culture by shaking the broth tube lightly for five minutes. At the end of this time the block of culture is removed from the broth and spread with the culture side down over the surface of a sterile slant of SABOURAUD's agar. These slants are then incubated at room temperature for three weeks and observed for growth. The effective fungicide should kill these test organisms within five minutes.

Bacteriostatic activity of antiseptics is tested by means of the serum-agar plate method or the agar cup-plate method as described by REDDISH in U. S. Department of Agriculture Circular No. 198. Under these conditions test is made for penetration as well as inhibition of growth. Similar technique may be employed in testing for fungistatic activity of various kinds of preparations which are applied for long periods of time.

Ointments, salves, powders, etc. which are used in treating epidermophytosis may be tested for fungistatic activity as follows: Add 2 c.c. of sterile normal horse serum to 18 c.c. of SABOURAUD's agar, which has been melted and cooled to 45° C., and pour into 9 cm. PETRI dishes. After the agar has hardened, streak each plate with one of the test fungi, using a dry sterile cotton swab. The preparation being tested is then added to the surface of the agar. Ointments and salves are melted and streaked on the surface of the inoculated agar by means of a glass rod, and powders are applied to the surface with a small spatula. These are applied to but a small portion of the plate, the ointment along a streak about 1.0 cm wide and extending across the middle of the plate, and the powders applied to an area of about 1 cm square. The plates are incubated at room temperature for five days. If the preparation tested is fungistatic, there will be a clear zone in the medium around the area where it was applied. The extent of this clear zone shows the amount of penetration and the effectiveness of the fungistatic ingredient.

Liquids and jellies which are applied for long time in treating epidermophytosis are tested by the agar cup-plate technique. The serum agar is prepared as for the agar plate method but when it is poured, a vial 1.0 cm in diameter is placed in the center of the plate and left there until the agar has solidified, after which it is removed and the small cracks and crevices in the resulting cup are filled with two or three drops of melted agar. The entire surface of each plate, including the cup, is then inoculated with one of the above test organisms. After this has been done, 0.5 c.c. of the liquid or jelly being tested is placed into the cup, the plates covered with an unglazed clay top, and incubated at room temperature for five days. Penetration and fungistatic activity will be shown by a clear zone around the cup.

D. Yeasts and Yeasts-like organisms

(1) Mycology

WICKERHAM and RETTGER (1939) made a comprehensive *taxonomic study of Monilia Albicans* with special emphasis on morphology and morphological variation. *M. Albicans* is made the object of this study because of its wide distribution and consequent ease of collection, and because it is the only important facultative parasite in the genus. In the historical review of the first attempts at classification of the Monilias readers are referred to the articles of LANGERON & GUERRA (1938), SHREWSBURY (1934), JUNGHERR

(1933), Langeron & Talice (1932), and Benham (1931). Strains of *M. Albicans* were obtained from a variety of sources. Thirty-nine were isolated from the human vagina and four form human faces. Cultures obtained from other investigators included seven strains from sputa tuberculous patients, ten from lesions of poultry and several from miscellaneous sources. Tomato agar medium described by Rettger, Levy, Weinstein, and Weiss (1935) was used only for primary isolation of the yeast-like organisms and the bacterial flora from the vagina and faeces. Corn-meal agar was the principal medium employed to determine the morphology of the strains studied. Dextrose agar was used primarily in the maintenance of stock cultures. Modifications of the Dalmau (1929) technique for studying the morphology of fungi were employed exclusively in this investigation.

The fermentation reactions of the three most commonly occurring Monilias. *M. albicans*, *M. candida*, and *M. parapsilosis* gave unsatisfactory results. Although the original aim was not attained, the following conclusions were arrived at: (1) That the fermentation medium should contain a relatively small amount of nitrogenous material along with a relatively large quantity of carbohydrate, in order to prevent the formation of basic substances in amounts sufficient to mask the acid reaction. Reversion to the alkaline side was quite rapid at 37° C. (2) That an over-abundance of oxygen reduces the fermentation reaction and increases the production of the basic substances although too great reduction of oxygen supply likewise reduces intensity of fermentation. (3) That incubation of the fermentation tubes at 30° C. is preferable to either 37° C. or ordinary room temperature.

As a result of these preliminary experiments, the following standardized technique was evolved for the determination of the fermentative reactions of the strains of monilia employed in this study:

Employment of the Standardized fermentation technique. . . . The medium: The basal medium contained 0.3 per cent of Difco yeast extract and 0.3 per cent Difco peptone. It was adjusted to pH 7.0. 1 c.c. of standard brom thymol blue solution was added to 400 c.c. of the medium before autoclaving. Test tubes of 12—14 mm diameter were used with insert tubes of about 8 mm internal diameter and a length of approximately 7.5 cm. The final medium had a depth of approximately 8 cm.

The carbohydrates were sterlized separately. 20 grm of the test material were dissolved in 80 c.c. of distilled water, adjusted to pH 6.8—7.0, and sterilized at 12 lb. pressure for twelve minutes. 1 c.c. quantities of the carbohydrate solutions were added to 9 c.c. of the tubed basal medium. The tubes were tested for sterility by incubation at 30° C. for forty-eight hours.

The inoculum: This was prepared as follows: The organisms were plated on tomato agar, and the plates incubated at 30° C. for forty-eight hours. Colonies were picked and streaked on tomate agar slants in 12 mm tubes. At the same time streak preparations were made for the morphological study, as described in a previous section. The slants were incubated at 30° C. for twenty-four hours. Material from the surface growths was emulsified in 2 c.c. of physiological saline solution, and 0—1 c.c. of the emulsions pipetted into each of the fermentation tubes.

The fermentation tests: Dextrose, galactose, maltose, sucrose and lactose were used for the indentification of species. The fermentation tubes were incubated at 30° C. and examined daily. The reactions became diagnostic in from forty-eight to ninety-six hours.

Consistent fermentation reactions can be obtained when the amounts of carbohydrate, nitrogenous material, and molecular oxygen supplied are optimal. The best results followed the filling of tubes to a depth of approximately 8 cm with a medium containing 2.0 per cent carbohydrate, 0.3 per cent yeast extract, 0.3 per cent peptone, and bromthymol blue indicator.

A detailed morphological study indicated that all strains have some characteristics in common, and that some strains posses properties that are peculiar to them. Environmental factors also play a prominent role in determining the morphology of this species, and affect different strains to varying degrees. It is believed, therefore, that classification of Monilia should be based on a broad concept of the morphological characteristics of this species, rather than on slight morphological details.

Strains of this species were arbitrarily divided into five overlapping morphological types, according to the comparative number of chlamdospores and blastospores produced when they were grown on cornmeal agar. The reactions of representative members of the five types to various conditions, such as variations on oxygen tension, age, medium, and temperature, were ascertained.

An abundance of available oxygen retards production of hyphae, stimulates the growth of blastospores and generally diminishes the production of chlamydospores. Where the oxygen supply to young hyphae is sufficiently reduced the hyphal cells persist as cytologically young cells. A slight reduction of oxygen tension is conductive to optimum fermentation reactions and to the best morphological development of the fungus, in so far as diagnosis is concerned.

On rich media, like dextrose or tomato agar, the ten representative strains produced no aerobic hyphae, and indeed few "anaerobic" hyphae. The number of chlamydospores was also reduced.

Incubation of the 10 strains at 30 and 37° C. inhibited the formation of chlamydospores. In certain strains the tendency for blastospores to develop in clusters was lost, and these bodies were formed at all points along the hyphae. Blastospores produced in this manner were often twice as large as normal.

The generally accepted belief that the Monilia genus is primarily unicellular in nature is shown to be untenable by the demonstration that this fungus may produce true hyphae which arise by septation of the filament, rather than by a succession of polar buds, and that a division of labor exists among the cells of the plant, as is shown in the utilization by developing chlamydospores of food produced in and transported from the hyphal cells.

Floriano de Almeida and Rosa Abdalla investigated some species of the *genus Torulopsis*. There is a group of fungi in mycology with an uncertain systematic position to which belong Monilia, Cryptococcus and Torulopsis. Ciferri and Redaelli placed them into the super family Atelosaccharomycetaceae of the Fungi Imperfecti, with three families i.c. *Histoplasmaceae*, *Nectaromycetaceae*, and *Torulopsidaceae*. The last family contains three tribes: *Torulopsideae*, *Mycotoruleae* and *Trichosporeae*. Dodge, on the other hand, put the genus Torulopsis into the family Saccharomycetaceae Imperfectae of the Fungi Imperfecti revealing intimate relations with the family Saccharomycetaceae Perfectae of the order Endomycetales of the class Ascomycetes.

Fungi of the genus Torulopsis were found in the expectoration of individuals with pulmonary lesions, tuberculous or otherwise, as well as in different cutaneous lesions. In the present investigation the authors, without discussing the pathogenic value of these fungi, make a short summary of these microorganisms, their process of study and classification. They then present the results of the macro-and microscopic study they made with six strains. In the study of these fungi, the authors state, nothing has yet been done in Brazil.

Roger D. Baker compared the infection of mice by *mycelial and yeast forms of Blastomyces dermatitidis.* It is known that organisms causing human blastomycosis are regularly found in the yeast form in the lesions; and in the mycelial form when grown on artifical medium at room temperature. The mycelial form can be transformed into the yeast form by animal passage or merely by growing on blood agar at incubator temperature. Repeated mouse passage does not appear to enhance the virulence for mice of the causative organism. The mycelial form of a strain of *Blastomyces dermatitidis* was found to be as effective as the yeast form in causing death of mice and extensive lesions in the abdominal cavity and lungs when weighed equivalent dosages were given intraperitoneally. The aerial growth of the mycelial form was as effective as the subsurface growth of the mycelium. When examined at the spontaneous death of the animals the lesions always contained the yeast form even when the mycelial form has been injected.

Hiromiti Takaoka made statistical observations and mycological investigations on Blastomyces in eczema and in all cases admitted to the dispensary the last eleven years. The frequency amounted to 0.2 % among 38140 dermatoses; men were affected six times oftener than women. The condition howed up most frequently between 51 to 60 years of age, it was relatively frequent between 41 to 50 years of age, and very seldom in the childhood. Seasonal difference were also found, with the highest morbidity in spring and summer. Cummulative occurrence was observed in agricultural vocations (29.4 %). Furthermore 22.63 cases of eczema from 31 years revealed 0.64 % eczema ani of all sick and 2.7 % of those afflicted with eczema. The mycological examination yielded a series of yeast strains, pathogenic or apathogenic, cultured from perianal scales, saliva and stools. Due to this fact that oftener the same "pathogenic" strain of yeast was found at the beginning and at the end of the digestive tract (saliva . . . stool . . .) it is inferred that yeast may sometimes causa anal eczema. The test strains are classified in three groups: Myceloblastanon (53.8 %), Cryptococcus (38.5 %) and Mycoderma (7.7 %).

Here again it must be with emphasis pointed to the superficiality of statements and assumptions which attribute pathogenicity to microorganisms . . . yeasts, bacteria or hyphomycetes . . . for the sole and exclusive reason because they were caught in a culture tube. The reviewer's large cultural experience concerning the microbiology of "anal eczema" consistently showed a great variety of microorganisms of all three varieties mentioned in different combinations. Yeasts and yeast-like bodies are permanent inhabitants of the oral cavity. We continuously take them in with various kinds of foods (raw fruits, beer, wine, etc.). The reviewer's investigations as to yeast and yeast-like bodies of the oral cavity revealed that in any group examined one species or biological strain is oftener represented than any other of the rest. They are swallowed, consequently they can be recovered with ease in the stools. Common molds (their spores) are also present in the oral cavity. Whether they pass the digestive tract undamaged as yeasts do appearing at its anal outlet or they adhere to the anal ring due to exogenous transportation, does not make any difference. Needless to refer to the presence of bacteria particularly in the lower part of the digestive tract.

Due to this ubiquity of microorganisms along the digestive tract we have to require the most complete proofs of their assumed pathogenicity and causative role in any cutaneous affliction. The proofs must be as thorough and complete in mycology as we are used to it in the bacteriological field. The reviewer could not convince himself in any case of "anal eczema" in a long experience that microorganisms play any role as causative agents.

MACKINNON and RODRIQUES-GARCIA investigated experimentally the *pathogenic action of Monilias.* Among the experimental animals, rabbits, guinea pigs, rats and mice, inoculated with 27 different strains of *Mycotorula,* mice were the most sensitive. The best ways of inoculation were for rabbits the intravenous route, for mice and guinea pigs the intraperitoneal way and intratesticular in rats. The histological reactions to the inoculated *Mycotorula* were quite different. Most of the strains had a high virulence which seemed to be rather constant.

COCHET reported on a new fungus, *Arthrographis Langeroni* n.g., n.sp., as a pathogen in human onychomycosis. This new species was isolated from a "typical clinically" onychomycosis. The culture yielded a slow growing colony with radial furrows and elevated centrum. The surface was yellowish, the center humid-glistening, and powdery at the periphery. Thallus consisted of arthrospores with true mycelium formation in addition. The mycelia ended in a brush-like structure (similar to the Penicillium) which split up again in arthrospores. The fungus is pathogen for rabbit and guinea pig. The dermal lesion is a superficial benign dermatomycosis healing spontaneously within a few weeks.

The chapter "onychomycosis" is one of the most confused ones in the whole medical mycology. This chapter ought to be urgently revised. Whensoever a new species of yeasts or yeast-like organisms, or new hyphomycetes are caught in a test tube culture from nail debris or detritus masses around or from below the nail plate, the authors are inclined to assume with certainly they found the causative organisms of the nail condition in question. When, in addition, as in COCHET's case, the new species proved to elicite some superficial, self-limited skin lesions in common experimental animals, then they believe to have done everything to infer the pathogenicity of the fungal organisms. This superficial way of thinking is most erroneous and leads to nothing but fallacies.

Real, true and proven cases of onychomycosis are extremely rare. There are few authenticated instances of favus and trichophyton infections. Beside traumatic injury of the nail plates, like in certain trades and professions, an overwhelming percentage of the nail affections is primarily the pompholyx of the matrix of the nail and the nail bed, a hemotogenous-endogenous affair. It is easily conceivable that bacteria, yeasts and molds intrude and settle down in the loosened structure of the damaged nail plate. There is no objective and acceptable reason and evidence to assume the pathogenicity of microorganisms vegetating in detritus material of a nail plate altered by other basic condition. And to support the assumption of simply saprophyric invaders by the evidence of the pathogenicity of the microorganisms found for experimental animals is naive and without any weight. If such a microorganism is found pathogen for laboratory animals, its pathogenicity is purely a biologic characteristic for the microorganism in question but is no evidence for its pathogenicity to human tissues.

W. H. KELLEY investigated the cell and colony variations of Blastomyces dermatitidis. The concentration of agar upon which Blastomyces dermatitidis is grown influenced to a noteworthy degree the cell and colony characteristics. Growth was more rapid with 1—2 % than with greater or lesser concentrations of agar in the beef-extract medium used. Optimal growth of the fungus in these experiments was obtained on beef extract medium containing 1.5 % agar, 1 % starch, and 5—10 % of fresh animal serum, when the pH was adjusted to 7.2—7.3 and the cultures incubated at 37.5° C. in a humidor. Under those conditions the fungus grew in the yeast-like form to produce colonies resembling those of the smooth mutant bacteria and yeasts. These findings permit certain analogies between the cell and colony variations of *Blastomyces dermatitidis* and those which characterize mutation in bacteria.

Mickle and Jones (1940) investigated the *dissociation of Candida albicans by lithium chloride and immune serum.*

The media used were as follows: Eighteen freshly isolated cultures of Candida albicans, 14 strains from the vagina and 4 from sputum, were cultured in lithium chloride or immune rabbit serum to induce dissociative changes. Fourteen of these strains produced variants which seemed permanent and differed from the parent from in gross and microscopic morphology and pathogenicity. It was concluded that these variants were true rough phases of *Candida albicans* and were analogous to dissociants that occur among the bacteria.

Summary of characteristics of smooth and rough forms

	Smooth	Intermediate	Rough
Sabouraud's agar	Smooth, creamy. Rounded up colonies	Smooth, creamy	Flat, dry, spreading. Colonies larger than smooth
Blood agar	Small gray creamy colonies	White, stellate creamy colonies	Dry, flat, irregular borders
Sabouraud's broth	Homogenous sediment	Homogenous sediment	Liquid clear. Extensive surface growth
Microscopic morphology	Round or oval budding cells	Oval or irregular cells	Large, elongated and irregular cells
Mycelial formation	Branching, tree-like. Numerous chlamydospores	Same as smooth	Brush-like, no chlamydospores
Carbohydrate fermentation	Glucose AG; Maltose AG; Surcrose A; Lactose O	Same as smooth	Same as smooth. Somewhat slower
Pathogenicity	1 ml of a 1 per cent suspension killed rabbit, 2—5 days	Required 2 ml. of a 1 per cent uspension to kill rabbit, 2—5 days	Not pathogenic
Agglutination	Marked cross agglutination of rough and smooth forms. Intermediate not tested		

Sabouraud's glucose agar. Bacto-glucose 40 grams, Fairchild's peptone 10 grams, agar 25 grams, distilled water 1000 ml. Melt in autoclave, filter through gauze, tube and autoclave at 15 pounds pressure for 15 minutes. No pH adjustment necessary.

SABOURAUD's glucose broth. Same as SABOURAUD's glucose agar, with the exception that no agar is added. No pH adjustment necessary.

Beef extract agar. Difco beef extract 3 grams, sodium chloride 5 grams, Difco peptone 10 grams, agar 25 grams, distilled water 1000 ml. Melt in autoclave, filter through gauze and titrate to pH 7.6. Tube and autoclave. Final pH 7.4.

Beef extract blood agar. Same formula as beef extract agar, with the addition of approximately 10 per cent sterile citrated sheep blood.

Carbohydrate broth. Same basic formula as beef extract agar except that no agar is added. After dissolving, heat to boiling and titrate to pH 7.2. Add 100 ml of indicator (below), tube and autoclave. Final pH 7.0.

Indicator solution. Brom-thymol-blue 0.04 gram, distilled water 100 ml. Titrate to the point that 1 drop of 1 N NaOH or 1 N HCl will cause a complete change in color.

Corn-meal agar. Corn meal 62.5 grams, water 1500 ml. Heat to 60° C. for one hour. Add 19 grams of agar and melt in autoclave, make up to 1500 ml with water, filter through gauze, tube and sterilize. No pH adjustment necessary.

MACKINNON (1940) carried out interesting studies on *dissociation in Candida albicans.*

In *Candida albicans,* spontaneous variations in two directions were observed, corresponding to two series of variants which have been called membranous variants and lethal variants.

Variation in the membranous direction is characterized by the elongation of the blastospores which become filaments (pseudofilaments). This fact brings as a consequence gradual modifications in the macroscopic appearance of colonies (in Sabouraud glucose agar). These, first, are surrounded by a filamentous halo; in more advanced variants, they become wrinkled, then they grow opaque; and finally, in extreme variants, they become spiky and hard. At the same time, in liquid medium (glucose broth), a mucous veil is observed and afterwards, in advanced variants, a membranous veil appears. The virulence diminishes. The filaments agglutinate in large clots in saline and trypaflavine solutions, but the blastospores of the membranous variants agglutinate in small clots as in the normal form.

The experimental virulence, high in the creamy normal form (first degree of virulence), is diminished in the membranous forms (first or second degree). Partial reversion to the normal form has been noticed. However, the reverted forms are instable and tend to return to the membranous form.

The biochemical properties do not suffer qualitative changes.

The other type of variation, the lethal variation, is characterized by a lower rate of growth by great diminution or total loss of virulence and by increasing difficulty to produce mycelial growth. The membranous and the lethal variations can be produced in the same strain giving origin to variants which combine lethal and membranous characteristics. A strain which has varied in the lethal direction can later vary in the membranous direction and vice versa. The membranous variant of *Candida albicans,* which were described in 1936, is homologous to the variants in several true and nonpathogenic red yeasts observed principally by LEPESCHKIN, GUILLERMOND, NADSON (wrinkled cultures), PHILIPPOV, SEPILLI and GUISO (mycelial phase or R form) RUNKARI and HENRICI (wrinkled variants), FABIAN and MCCULLOUGH (R form) and KRASSILNIKOV. It is identical with the R form described in C. albicans by BESTA in 1938 and CAVALLERO in 1939. The R form described by NEGRONI is identical with the lethal form which has been described in this paper. It is thought that the races that KRASSILNIKOV and NADSON have created form *Saccharomyces cerevisiae* and which they have called "lethal races" are homologous to the lethal variant of *C. albicans*.

As it was pointed out, some of the strains of *C. albicans* differ frome one another by more or less pigmentation, and it is believed possible to detect, as in red yeasts, variation characterized by discoloration. This variation is probably occurring in combination with the two already described. *Candida albicans* is therefore a protean species. The number of possible combinations of characteristics which this species can produce is theoretically infinite. It is easy to understand why it has been given so many different names. The study of hereditary variations is necessary to identify a species of fungus. Variations must be studied methodically in all the pathogenic species in order to discover all the possible directions of variation, and, in consequence, to enable us to predict the forms which it can present. This method will help to improve in the identification and classification of pathogenic fungi and then eliminate the existing chaos and confusion.

CASTELLANI published a brief note on a strain of *Monilia* (*Candida*) *Zeylanoides*, Cast., isolated form a case of Moniliasis of the toes. The "Monilia Zeylanica group" contains the following chief species: (1) *Monilia* (Candida) *Zeylanoides* Cast., 1916, (2) *Monilia* (Candida) *deformans* Fach, 1934. *Monilia* (*Candida*) *Zeylanica* is characterized by producing pigmented yellowish colonies on glucose agar and by clotting the milk. *Monilia* (*Candida*) *deformans* produces white colonies on maltose agar and glucose agar; it does not clot milk and does not liquefy gelatine. It contains strains which differ among themselves with regard to the presence or absence of acidity in glucose and other sugars, presence or absence of pigmentation in arbutin agar. A strain isolated from a case of mycotic macroglossia, which does not acidify any sugar has been described as Monilia Zeylanoides var. macroglossiae. The original strain of *Monilia Zeylanoides* was lost long ago.

The following is a simple key for the identification and determination of the species and varieties belonging to the *Zeylanica-Zeylanoides group* of Monilias: (A) Yellow or white-yellowish colonies. Milk coagulated: (a) Gelatine rapidly liquefied, Deformans; (b) gelatine not liquefied (during first three weeks), Zeylancia. (B) white colonies. Milk not coagulated. Gelatine not liquefied: (a) glucose acidified, Zeylanoides; (b) glucose not acidified, Zeylanoides, var. marcroglossiae.

The *Monilia* (Candida) *Zeylanoides* strain isolated from case of toe Moniliasis has the following characteristics.

Dimensions. — The fungal cells (blastospores vary in size between 4.5 × 6 μ and 1 × 2 μ, most of them being 2.6 × 4 μ. In the condensation water of glucose-agar cultures much larger forms may be noted, 4.5 × 7.5 μ.

Biochemical characters. — Gelatine is not liquefied (period of observation three weeks); coagulated serum is not liquefied (period of observation three weeks); litmus milk shows no change the first few days, then it becomes slightly alkaline; there is no clotting and no peptonization (period of observation three weeks). There is no production of gas in any of the sugars and other substances tested. Glucose, levulose, maltose, galactose, saccharose, inulin, are rendered acid (no gas) within a week. The acidity in maltose, galactose, saccharose, may be very slight. No change in lactose.

Arbutin agar. — Deep brownish-black pigmentation of the medium is noted in cultures a week old kept at 30° C.

SCHNOOR investigated the *occurrence of Monilia in normal stools.*

A series of 314 normal stools has been cultured to determine the frequency of fungi in the gastro-intestinal tract of normal man. *Monilia* was found in 33.1 per cent of the stools cultured, of which 16.9 per cent were *Monilia albicans*; 6.0 per cent *Monilia parapsilosis*; 5.7 per cent *monilia krusei*; and 4 per cent *Monilia candida.* A significant number of colonies of Monilia (more than 12 colonies per plate streaked with a 4 mm loop) was found in only 6 of the 314 specimens (1.9 per cent), and by the same standard *M. albicans* was found in only 3 specimens (1 per cent). Other fungi isolated and identified were: *Cryptococcus* 28 per cent, *Geotrichum* 29 per cent, *Penicillium* 11.8 per cent, and *Aspergillus* 3.5 per cent.

KNAYSI (1940) reported on *observations on the cell division of some yeasts and bacteria.*

The process of cell division of the yeast *Schizosaccharomyces pombe* and of a number of common bacteria was investigated in dark and bright fields. The dark-field observations were made on a number of bacteria including *Bacillus cereus, Bacillus subtilis, Bacillus mesentericus, Bacillus magatherium, Bacillus aterrimus, Bacillus niger, Aerobacter aerogenes, Proteus vulgaris, Streptococcus fecalis, Mycobacterium tuberculosis* and the yeast, *Schizosaccharomyces pombe.* BRIGHTFIELD studies were made on *Bacillus cereus, Aerobacter aerogenes, Proteus vulgaris, Streptococcus fecalis* and *Schizosaccharomyces pombe.* In the latter case a new way of making the cell-wall visible is given. The observations indicate a fundamental difference between cell-division of yeasts and bacteria. In yeasts, there are no dark-field indications of cell division until it is completed. In bacteria, a break in the cytoplasmic membrane presages cell division. Bright-field observations show that the depositon of cell-wall material precedes the breakup of the cytoplasm in yeasts and follows it in bacteria. In both groups two walls are deposited form the beginning. In the organisms studied, there was no indication of the formation of a single wall with subsequent splitting, not of division by simple constriction unless this is applied to the cytoplasm alone.

MARTIN and JONES (1940) published *further studies on the practical classification of the monilias.*

Five hundred and thirty-five strains of non-ascus-forming, mycelia-producing, yeastlike fungi were studied. (MARTIN, D. S., JONES, C. P., YAO, K. F., and LEE, L. E. 1937 A practical classification of the Monilias. J. Bact., 34, 99—128). Of 461 recently isolated strains, 457 (99.13 per cent) agreed in all details with the criteria established for the previously described 6 species. Forty-six of the 73 previously named strains were likewise identified as members of the previously described 6 species. Sixteen of the remaining previously named strains were tentatively classified as belonging to the previously described species but differed from typical strains in some detail (blood agar colony or mycelium production). Four strains were apparently mislabeled as they did not correspond to the original description or to the descriptions of other workers. The remaining 8 strains did not fit into the classification previously proposed and are described under the heading "other strains". The great majority of freshly isolated non-ascosporogenous mycelia-producing, yeastlike fungi (the Candidas) can be identified by the procedure formerly described from this laboratory. Cultures which have been kept on artificial media for many years become dissociated or changed in such a way that they are no longer typical and, therefore, cannot be used for comparison in the identification of recently isolated strains. Slight variations in technique of study produce such great changes in growth and biological characteristics that many of the older descriptions must be interpreted with great care before arriving at definite opinions concerning their position in more modern systems of classification.

JOACHIM and POLAYES (1940) observed the first case of a *subacute endocarditis* with *systemic mycosis* due to *monilia* recognized during life.

A white man aged 48, American born, had been addicted to the use of morphine and heroin for twenty years. For eighteen months he had injected the drug intravenously. No aseptic or antiseptic caurions were observed. The patient averred that in January 1939 he ceased the use of the drugs on account of his financial inability to secure them and submitted himself for the treatment of his drug addiction. He suffered from withdrawal symptoms such as salivation, joint and muscle pains, abdominal cramps and diarrhea. These symptoms persisted and he was treated for "rheumatism". He began to lose weight and became progressively weaker. Fever developed.

A total of eleven blood cultures were made. The first, on May 3, and each subsequent culture showed the presence of innumerable colonies, of yeastlike cells. Throat, stool and urine cultures failed to show any fungus. Cultures of the contents of the container from which the drugs were injected showed organisms identical with those obtained from the blood. The vein into which the drug was injected revealed the following features:

On microscopic examination the skin and subcutis showed numerous deposits of iron-free, nondescript particles and fibrosis. Many of the particles were being engulfed by foreign body giant cells or surrounded by a reactive inflammatory zone, the infiltrates of which consisted predominantly of mononuclear and plasma cells. Occasional yeastlike cells were demonstrable among the infiltrating cells and in the interestices.

Bacteriologic Description of the Organism. — Morphology: The organism was ovoid and averaged about 4 microns in diameter; there were atypical short mycelia. One of the Sabouraud subcultures showed distinct hyphae.

Cultural Characteristics: Nutrient, dextrose and blood agar mediums showed opaque yellow convex round nonhemolytic colonies after from forty-eight to sevety-two hours. The temperature of incubation was 37.5° C.

Stab culture on SABOURAUD's medium yielded an inverted fir tree type of growth. In broth there were heavy sediment and slight cloudiness of supernatant fluid after seventy-two hours incubation. In gelatin no liquefaction occurred.

Sugar reactions were as follows: There was no acid or gas in lactose and mannite. There were acid and about 10 per cent gas in maltose, saccharose and dextrose.

The provisional diagnosis was moniliasis.

Serologic examination. — Agglutination test performed with the fungus recovered from the blood culture and the patient's serum gave the following reactions:

Serum dilution 1: 10, marked agglutination in five minutes.

Serum dilution 1: 100, definite agglutination in twenty minutes.

There was no agglutination with saline solution or with normal human serum control.

The patient's serum agglutinated known cultures of Monilia albicans to a dilution of 1: 640.

LAFFER (1940) published *a note on Saccharomyces fragilis* JORGENSEN *associated with pathologic conditions in human beings.*

Saccharomyces fragilis was first isolated by JORGENSEN from kefir, and has repeatedly been found in dairy products. Within the last fifteen years, there have been two reports of the isolation of yeastlike fungi from pathologic conditions in human beings in which the organisms proved to be *Saccharomyces fragilis* Jorgensen. In both cases, the organisms were first classified as some other species, but upon detailed study at the Centraalbureau voor Schimmelcultures at Baarn, Holland, they proved to be strains of *Saccharomyces fragilis.* In the first case, REDAELLI in 1925 (STELLING-DEKKER, 1931) reported the isolation of *Saccharomyces cavernicula* n. sp. from the lung of a tuberculous patient.

In 1935 CIFERRI and REDAELLI (DIDDENS and LODDER, 1939) described a species that had been isolated by Carco at Catania from a lesion of the human tonsils and pharynx.

Case history. The patient, a male, came to the physician with an acute infection of the tonsils, characterized by a sore throat, fever, malaise, anorexia, fetid breath, terribly coated tongue, headache, backache, and pains in the limbs. White or yellow patches of exudate were present in follicular form. Intensive treatment for follicular tonsillitis in the first seven days did not show the slightest improvement. On the eighth day he was given 10,000 units of diphtheria antitoxin, and the dose was repeated on the ninth day; he recovered rapidly without further treatment. At no time were there objective signs of diphtheria.

1. A third case in which *Saccharomyces fragilis* Jorgensen has been isolated from a pathologic condition in human beings is reported. 2. Confirmation of the relationship of *Saccharomyces fragilis* Jorgensen and *Candida* (Monilia) *pseudotropicalis* (Castellani) Basgal, as indicated by DIDDENS and LODDER (1939), is implied. 3. The desirability of an attempt to further confirm this relationship by serologic studies is suggested. 4. While nothing is contained to indicate that *Saccharomyces fragilis* is the causal agent in the infections of the throat, the finding of this species in throat cultures might prove worthy of a more detailed investigation or might prove to be a lead worthy of following. Careful morphologic studies of yeasts found, including repeated attempts at ascospore determination, and the inoculation of lactose, sucrose, inulin, maltose, litmus milk, and gelatin, would probably suffice for preliminary study and tentative identification. The wide distribution of this organism is clearly shown. This species was found in cream obtained in Illinois, and has been identified as *Saccharomyces fragilis* cultures (from milk; from yoghurt; from sour mil and whey. It is easy to see how such organisms could get into the throat and thus be found in examination of throat cultures.

ALMEIDA and SILVA LACAZ (1940) isolated a fungus of the *genus Saccharomyces* from the sputum of a woman with parenchymatous pulmonary lesions. Mycobacterium tuberculosis was negative. It is a rarity, indeed, to isolate members of *genus Saccharomyces* from human pathological products.

NEGRONI and FISCHER (1941) investigated the *Biology* of "*Vahlkampfia debilis*" *Jollos*, 1917, an ameba parasitized by a yeastlike organism and the phenomenon of lysis resembling bacteriaphagia.

The authors describe the saccharomycetic and bacterial lysis produced by *Vahlkampfia debilis* Jollos, 1917. The characteristics of this lysis are comparable to those produced by bacteriophage. This curious phenomenon took place spontaneously in nature in an apple, still attached to the tree, invaded by yeast and ameba. They believed that the yeast developed first and then the ameba which has been carried by birds or insects. The morphological and biological characteristics of this ameba are those of the genera *Vahlkampfia with* the following characteristics: vegetative cells are from 8 microns to 49 microns in diameter with an average of more or less 15 microns. Endoplasm and ectoplasm well differentiated. Each ameba has only one nucleus of about 3 microns in diameter. Glycogenic reserves and flagellate stage are lacking. Cysts: oval, elliptical or less frequently globose, from 6,12 microns to 11,47 microns × 7,65 microns in diameter with a smooth, thin membrane. Biological characteristics: This, ameba grows abundantly in our liquid or solid culture media provided that living yeasts or bacteria are present. They have tested the lytic power on more than 60 different species of yeasts, on filamentous fungi and on bacteria. They succeeded in modifying at will the internal flora (feeding inclusions) of the ameba.

The different species of yeasts offer a gradation of sensibility to the lytic action of *Vahlkampfia debilis*. The yeast isolated from the original material was classified as *Pichia belgica* and it is as well phagocytosed as *Candida aldoi*, *C. deformans* or *C. zeylanoides*. On the contrary, *Candida albicans*, the filamentous fungi (Actinomyces, Mucedinaceae and Dematiaceae) and gram positive bacteria are not attacked. The gram negative bacteria of the typhoid-dysenteric group are easily phagocytosed. The lytic action begins at the edges of the colonies and advances towards the center. At the end of 3 or 4 days a lacunar halo is formed around each colony; consisting of pure growth of vegetative ameba. Nevertheless the yeasts or bacteria do not disappear completely and a biological equilibrium is established between the ameba and its host. A lytic period is followed by a period of secondary development of the yeast cells, and this in turn by another lytic period, this phenomenon is repeated several times. The ameba is unable to digest the membrane of yeast cells or to inclose starch grains but it is able to phagocytose human or rabbit red blood corpuscles.

Optimum temperature for growth is 30° C. The cysts begin to appear after 2 or 3 days. The ameba keeps its vitality for 2 or 3 months at room temperature in culture media and still longer if dried in phosphoric vacuum and kept in the ice-box. This ameba withstands a temperature of 0°, a wather-bath at 45° for 20 minutes, or the action of a 20 % citric acid solution. It develops in synthetic media with 3 % of ethylic alcohol. Vegetative cells are quickly destroyed by fresh calf bile. Pathogenicity for young cats is negative. Agglutination and complement fixation tests with sera of immunized rabbits are positive. Intracutaneous inoculation of an acqueous extract of the ameba in immunized rabbits produces a red papule of about one inch in diameter (allergy).

They call the attention of the protozoologists to the peculiar characteristic of the vegetative ameba of ingesting its own cysts and especially to the curious multiplication by transverse septation of the cysts which occurs chiefly in liquid media.

POLAYES and EMMONS (1941) published a *final report on the identification of the organism of the previously reported case of Subacute Endocarditis and Systemic Mycosis* (*Monilia*).

In the report of the first case of subacute endocarditis with systematic moniliasis ever to have been clinically diagnosed and published (JOACHIM, HENRY, and POLAYES, S. H.: Subacute Endocarditis and Systemic Mycosis (Monilia), J. A. M. A. 115: 205 (July 20) 1940, it was not possible to state the identity of the causative organism, although it was definitely known to be a species of *Candida* (Monilia). Growth on SABOURAUD's agar yielded budding ovoid bodies about 4 microns in diameter; hyphae were very rare; cornmeal agar yielded budding ovoid cells from 3 to 4 microns in diameter on the surface; hyphae below the agar surface were delicate, short and branching freely but producing few buds. On staining, the yeasts were gram positive except for an occasional pink hypha. Reproduction was by budding. Growth characteristics were as follows: In acid dextrose broth after forty-eight hours at 37.5° C. there was growth settling to the bottom and clinging to the side of the tube; the broth was clear except in a few tubes. There was no ring or the ring was barely noticeable at an angle formed by the broth surface and the side of the tube. On acid dextrose agar after ten days at 30° C. the colonies were ivory to yellow and glistening with margins smooth or slightly scalloped. On blood agar after ten days at 37.5° C. culture yielded gray, smooth, glistening, nonhemolytic, low dome-shaped colonies with even borders from 2 to 3 mm in diameter. Sugar reactions were as follows: There were acid and gas formations in dextrose and levulose broths but not in sucrose, lactose, maltose and raffinose. The amount of gas formation varied, only small bubbles appearing in most tests.

BURT and KETCHUM (1941) reported on the *Classification of strains of Candida (Monilia) isolated from sputum.*

The sputa of 693 sanatorium patients have been studied and 250 strains of the genus Candida isolated, an incidence of 36 per cent. Of these 205 (82 per cent) have been classified as *C. albicans,* 40 (16 per cent) as *C. tropicalis,* 2 (0.8 per cent) as *C. Krusei* and 3 (1.2 per cent) strains remain unclassified. Fifteen cultures from other laboratories have also been compared using the same method of study. By inoculating a plate containing malt agar and modified DURHAM tubes containing sucrose and maltose the great majority of strains isolated from sputum can be classified. The procedure is, therefore, simple and the media are easily prepared. Other methods should be resorted to if this plan fails. The generic term Candida has been substituted for Monilia. Different names for some of the species have also been used.

(2) Clinic

KNIGHTON (1939) made a study of *Monilia and other yeastlike organisms found in the oral cavity.*

A review of literature reveals the widespread occurrence of yeastlike fungi. The lack of a standard method of classification has caused much confusion in the study of these organisms. More recent attempts to simplify the classification have been beneficial in studying yeastlike organisms both in normal and in pathological conditions. It is important to note the impossibility of dividing the organisms into their correct genera by morphological study alone. The aim of the experiments reported in this work was to study yeastlike organisms found in the oral cavity using the more recent methods of classification.

Initial cultures taken from the mucous membrane of 146 oral cavities were positive for yeastlike fungi in 36.9 per cent of the cases and for *Monilia albicans* in 23.9 per cent. Yeastlike fungi were found in 32.5 per cent of 123 dentate cases and in 60.87 per cent of 23 cases either fully edentulous or wearing partial dentures. The percentage of cases positive for *Monilia albicans* was 24.39 per cent for the dentate and 21.74 per cent for the edentulous series. Therefore, it is evident that, in this study, yeastlike organisms other than *Monilia albicans* are found with greater regularity in cases wearing artificial dentures.

In a study of the incidence of yeastlike organisms in the same mouths over a series of weeks, the organisms were considered as constant inhabitants in 4 of 26 cases (15.4 per cent). The yeastlike fungi harbored in the four mouths were *Monilia albicans.*

The only case, in the series of 26, wearing a partial denture had constantly positive cultures for a Cryptococcus in addition to the *Monilia albicans.*

Yeastlike organisms found occasionally in other mouths studied over a series of weeks were relatively evenly distributed among Monilia, Cryptococcus, and Saccharomyces.

In a study of different areas of mouths known to harbor yeastlike fungi the organisms were found to be scattered over the mouth but the tongue and throat cultures were the most constantly positive.

The percentage of positive cases agree in general with FISHER's (18) work. The finding of organisms scattered throughout the mouth is also in agreement with FISHER (18), who found *Monilia albicans* in the saliva of 26.2 per cent of the cases. The percentage of positive cases was greater than the 14.7 per cent reported by TODD (19). This may possibly be explained by the fact that in the author's experiments swabs were cultured on slants and in broth.

In the series studied no lesions resembling thrush or perleche were noted. Correlation could be made between the presence of yeastlike fungi and the health of the gingival tissues. In a series including cultures from 50 carious teeth no correlation between caries and the presence of yeastlike organisms could be detected.

These experiments along with a review of literature suggests that yeastlike organisms may be found in apparently normal mouths both as transients and as constant inhabitants. Of the yeastlike organisms found *Monilia albicans* is most often encountered. Pathogenicity experiments prove that the *Monilia albicans* strains found in apparently normal mouths are capable of producing a generalized moniliasis in rabbits.

In discussing the role of Moniliae in bronchial and pulmonary infections, BENHAM (2) pointed out that it is debatable whether the organism found in the sputum is the causative agent, contributing factor, or an accidental finding. The oral pathologist is confronted with much the same confusing problem. The organisms are present in many apparently normal mouths without causing any perceptible pathological condition, yet one cannot completely disregard the yeastlike fungi because of the many reports of lesions in the oral cavity in which the organisms are suspected of being either the exciting or contributing cause.

The reports of CAHN (32) and BARTELS (34) are evidence of the etiological role Moniliae may play in denture sore mouth but one must not forget the many other possible causes of denture sore mouth: ill fitting dentures, systemic conditions, and possible sensitization to baseplate materials and denture creams.

The evidence at hand seems to justify the following conclusions: The mere finding of yeastlike fungi in oral lesions is not sufficient evidence to attach any etiological importance to the organisms. The fact that the organisms are found in many normal mouths does not license the oral pathologist and bacteriologist to disregard them if found in lesions. These points stress the importance of correlation of findings by the laboratory and clinical diagnosticians.

BENHAM (1939) investigated the cultural characteristies of *Pityrosporum ovale — a Lipophylic fungus.*

Since 1873 and 1874 when RIVOLTA (1) and MALASSEZ (2) first described the small ovoid cells which appear in scales from *Pityriasis capitis,* numberous reports on the cultivation of this organism and its etiological significance in seborrheic dermatitis and dandruff, have appeared. The bottle bacillus or spore of MALESSEZ is present in all or practically all dandruff scales. It appears varied in form, ranging from round to elongated cells, measuring 2—2.5 microns ×

3.5—4.5 microns, some being less that 2 microns in diameter. The organism is difficult to cultivate. As the organism lacks striking morphological characters and is not definitely pathogenic, it is difficult to prove that a fungus recovered in cultures from scales is identical with the form seen so abundantly on direct microscopical examination. The investigators mentioned based their conclusions on the similar morphology of the cells seen in scale and in culture; MOORE et al. (16), on the production of lesions on the human skin with their culture. There is difference in opinion as to its pathogenicity. Various yeastlike organisms as well as other fungi may be cultured from the scalp. This fact may be brought out by a study of the various cultures reported as being the bottle bacillus.

Of the cases studied, the bottle bacillus or pityrosporum was found to be present in the methylene blue preparation of the scales in 28. In such preparations the organism appears as small blue staining bodies, some dense, some vacuolated, of the characteristic round or elongated bottle shape, of an average size of 2 × 0.8 microns ranging from 0.8—1.5 × 2—3 microns.

A number of fungi such as species of aspergillus, penicillium, and alternaria were obtained as well as a variety of yeast-like organisms. These saprophytic fungi were most numberous on the tubes incubated at room temperature.

Cultural Characteristics. Attempts were made to culture the organism on a variety of media. Successful growth was obtained on PETROFF's egg medium plus gentian violet, white powdery colonies appeared. When the wort agar was replaced by Sabouraud's honey agar and the usual fatty substances added, a similar growth was obtained. When CURRIE's agar, a synthetic medium, was used as a base the growth was decreased. This medium has the following formula:

Sugar (dextrose)	50 grams
NH_4NO_3	10 grams
KH_2PO_4	5 grams
$MgSO_4$	25 grams
Asparagin	5 grams
Agar.	20 grams
Water	1000 cc.

The organism will not grow perceptibly on the CURRIE medium unless the fats are added. On the other hand, a slow, very minute growth may occur on the plain wort agar. The growth is very slight, the colony tiny, thin and transparent, looking much like a streptococcus colony. On the enriched wort medium the growth is much heavier. It appears first in the form of smooth, fine, cream colored colonies scattered over the surface of the medium. These may reach a diameter of several millimeters, remain distinct, or fuse, especially if the medium is moist, forming large plaques or masses of growth. This growth is usually dry and wrinkled in appearance and the color varies from cream to tan and finally orange brown. It is interesting that oleic acid, a fatty acid found commonly in nature, is a good source of food for this organism. No growth was obtained in the sugar broths.

Microscopic Characteristics. The organism characteristically has several forms in culture though usually it appears as an elongated structure 2—3 microns × 4—5 microns. It frequently appears two-celled, one of the cells of the pair being smaller, suggesting a bud. In some instances the bud has elongated forming a neck-like projection. This is the so-called "bottleform". Often the cells appear joined by a septum, indicating that the reproduction might be intermediary between budding and septation, as described for the genus saccharomycodes. These yeasts form buds which become separated from the parent cell by the formation of a septum at the base rather than by constriction of the base. This is a rare group and only one species is well known, *Saccharomycodes* (Saccharomyces) *Ludwigii*. This species forms ascospores and is much larger than the strain discussed here.

Sometimes the organisms lie in parallel pairs. The daughter cell, after separating from the mother cell, continues to lie near it. There is no indication of mycelial development and no multiple budding. Large eggregate masses form by the clumping of cells. Individual cells may contain small vacuoles. Some spherical thick walled cells are seen. These may be of the nature of resistant cells and are characteristic of most yeasts. Comparing this organism with the description given by OTA and HUANG for their cultures of *Pityrosporum ovale*, there seems little doubt that they are one and the same organism.

This organism is unlike the other cryptococci and probably belongs near the genus saccharomycodes though as yet no ascospores have been seen. It seems therefore to retain for it the name originally given and now so familiar in medical literature, *Pityrosporum ovale*.

Pathogenicity. Rabbits were inoculated by the method mentioned above with the strain of pityrosporum here described, as well as with Moore's strain and a strain of Group III cryptococcus from the feces of a normal individual. For the first few days after inoculation there was a scabbing over due to the scarification, after this the lesion cleared. There was some evidence of scaling in all cases, but no indication that one organism differed from the others. The organisms were not recovered in culture. The inoculations were repeated several times. Attempts were made to make the skin more favorable for the growth of the pityrosporum by rubbing the shaved area with butter for several days before inoculating. This also was without effect. As yet then, the evidence seems to be that the organism is non-pathogenic; and would seem to favor the view point that the organism is a saprophyte which finds favorable conditions for growth in the excretions from the scalp.

One must admit that the statements of MACLEOD and DOWLING and of MOORE et al, that they produced lesions resembling seborrheic dermatitis by inoculation of humans with their culture seems prima facie evidence that they recovered the causative microorganisms. The cultures obtained by these two groups of workers were, however, admittedly unlike and if one accepts this evidence it would prove that seborrheic dermatitis is caused by two quite different fungi.

Others have shown that vitamin B favors the growth of some yeasts and hyphomycetes. TOCHINAI and FERNI (35) on the other hand, have shown that vitamin A retards growth of certain hyphomycetes.

This tudy presents no evidence as to the etiological significance of the organism.

MARZOLLO (1939) reported on *Paronychia* and *Onychia* with peculiar *Discoloration* of the *Nail plates*, caused by *Cryptococcus interdigitalis*, POLLACCI and NANNIZZI.

The patient revealed an affliction of the nail plates of the index finger of both hands as well as of the nail plate of the middle finger of the right hand. The nail plates of the other fingers were absolutely normal. The paronychium of these fingers involved showed swelling, redness and discharged from underneath droplets of thick, creamy, yellowish-white matter. The nail plates of the involved fingers showed grooves and humps perpendicular to the long axis of the nail phalange. From the material discharged underneath the posterior nail wall a yeastlike organism was cultered and indentified as *Cryptococcus interdigitalis*, Polacci and Nannizzi (1926). The etiologic role of this organism was attempted to be proved by animal experiment, biologic tests (agglutination, complement fixation), by intradermo-reaction. The author believed to have proved the direct etiologic role of these yeastlike organisms in the elicitation of the onychia and paronychia.

EMMONS (1940) isolated and investigated the *Pathogenicity* of *Pityrosporum ovale.*

P. ovale was repeatedly and easily isolated by planting scales from seborrhea oleosa in acid dextrose broth containing 23 to 44 per cent glycerin and incubating at 30°—37° C. Subcultures were successfully carried on media prepared by pipetting ether extract of lanolin, oleic acid, or seborrheic scales over agar slants, as described by BENGAM. Evidence that the organism was actually *P. ovale* was furnished by the necessity for using special media, the resemblance of the fungus in culture and in the skin, and a series ob observations of the inoculum which showed a continuity of development of the cells of *P. ovale* in the scales.

ALMEIDA and SILVA LACAZ (1940) investigated *Yeastlike organisms* isolated from *sputum.* They examined the sputum in 422 pulmonary involvement. They found the sputums being

1.	positive for Koch bacillus	52 times
2.	,, ,, ,, ,, and fungi	19 times
3.	,, ,, ,, ,, and pneumococcus	2 times
4.	positive for fungi 140 Yeasts	110 times
	Aspergillus	14 times
	Penicillium	12 times
	Yeast & fungus	1 time
	Rhizopus	2 times
	Mucor	1 time
5.	Negative for Koch bacillus and fungi	209 times
		422 times

In the whole material 33,1 % were positive for fungi, 4,5 % for Koch bacillus and fungi and 12,3 % for Koch bacillus alone. The presence of yeastlike organisms in connection with pulmonary involvements was enormous reaching 78,5 % of all cases.

They suggested the following practical classification of yeasts or yeast-like organisms for generic diagnosis:

Blastosporales (cream-like colonies)	Yeastlike organisms without mycelia (true yeasts)	Ascus present (ascosporagenous yeasts)	*Saccharomyces Willia Debaryomyces* and others
Yeasts		Ascus absent (anascosporagenous yeasts)	*Torulopsis Rhodotorula* and others
	Yeastlike organisms with mycelia (false yeasts)	Ascus absent	*Candida*
Arthrosporales (pseudomembranous or membranous colonies)		*Geotrichum Neogeotrichum*	

Primo contributo alla conoscenza dei Micromiceti dell'Africa orientale [1]

di

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(12.XII.1949)

In questo contributo sono elencate le specie fungine determinate su una prima porzione del materiale che ho raccolto nelle regioni orientali dell' Africa: Scioa, Amara e soprattutto Galla e Sidama; regione quest' ultima in molte zone quasi nuova alla conoscenza micologica.

La vastità di questi territori, che lentamente dall' altopiano etiopico digradano verso il Sudan, e la conseguente varietà degli ambienti contribuiscono certo a rendere ancor più insufficienti le conoscenze già di per sè scarse che se ne hanno ed accrescono le difficoltà di chi voglia brevemente riassumerne le caratteristiche biologiche ai fini di una migliore descrizione di organismi in essi viventi.

La classificazione altimetrica in tre piani di vegetazione, solita anche tra queste popolazioni indigene („quollà'', „uoina degà'', „degà''), che sembra pur sempre attendibile in via generale, non può, dare utili insegnamenti a chi già non conosca le località così distinte, a meno di non volere attribuire ai limiti altimetrici del quollà (dai 600 ai 1700 m.s.m.), dell' uoina degà (dai 1700 ai 2000—2500 m.s.m.) e del degà (sopra i 2500 m.s.m.) un valore reale che essi non hanno; il che sarebbe causa di errori.

Il carattere delle formazioni vegetali, susseguentisi a grandi linee abbastanza regolarmente in funzione dell' altitudine, dalle steppe di Andropogoneae e dalle boscaglie megatermo-xerofile digradanti verso il bassopiano sudanese, alle foreste tropicali del Caffa, dei Masango, dello Uollega, e alle vastissime savane dell' altopiano, è nelle singole località non meno fortemente determinato dall' influenza più o meno prevalente dei regimi climatici sudanico od eritreo, dalla giacitura e costituzione dei terreni (terre argillose, caoliniche, terre rosse, lateritiche), dalla posizione rispetto alle località stesse delle grandi masse acquee della regione, dall' azione talora assai notevole dell' uomo sui consorzi vegetali. Nè è da dimenticare, particolarmente in queste regioni, l'influenza dell' accentuato periodismo stagionale, non solo sull' aspetto del paesaggio vegetale, con gli innumerevoli adattamenti che in esso induce, ma anche sulla natura della microflora fungina che vi staziona.

In genere, per quel che riguarda il materiale esaminato, sembra di poter notare l'influenza preminente dell' epoca, durante la quale le regioni sono state percorse, sulla qualità oltre che sull' abbondanza di esso. Ad esempio nel materiale che si elencherà, raccolto in periodo secco — dall' ottobre 1938 al maggio 1939 —, difettano le peronosporacee, di cui si è osservata solo *Plasmopara viticola* nei pressi di Addis Abeba (a circa 2350 m.s.m.) [1] e gli ifali mucedinei, se si eccettuino le forme conidiche di erisifacee, di cui è ben nota la resistenza a condizioni ambientali avverse. Mentre si è notata tra gli ascomiceti la relativa abbondanza, ad esempio sui *Ficus* e sui cereali in genere, specie in alcuni ambienti, delle forme a stroma dothideoide o phyllachoroide clipeato, ben protette dall' azione dei fattori esterni. E nei mesi immediatamente successivi al termine delle piogge — contraddistinti da più ingenti escursioni diurne dei fattori climatici — si è osservata la particolare frequenza delle ruggini, e specialmente delle forme uredosporiche, delle quali nel continuo succedersi e accavallarsi dei cicli vegetativi si può forse supporre la ininterrotta moltiplicazione nei vari ambienti. La presenza degli Ustilaginali anche in zone che sembrano avverse alla vita dei parassiti vegetali può forse essere attribuita all' indipendenza di questi miceti dall' ambiente esterno, cioè ai loro particolari modi di vita e riproduzione. Essi in dette zone si presentano difatti non di rado notevolmente dannosi ai cereali di più larga e tradizionale coltivazione (dura, grano, orzo).

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[1] Il presente lavoro, già pronto nella seconda metà del 1940, non potè essere portato a conoscenza degli studiosi per le condizioni di isolamento nelle quali venne a trovarsi l'Africa Orientale dopo l'inizio delle ostilità con i territori limitrofi.

Esso viene ora reso noto nella forma originaria, che, meno trascurabili varianti, è rimasta intatta.

Di ogni specie raccolta pertanto si sono indicati, oltre al luogo del reperimento, i bacini, i corsi d'aqua (laghi: Tana, Zuai, Margherita, Auasa; fiumi: Omo Bottego, Nilo Azzurro, Didessa, Ghibiè, Sachi, . . .) o nomi di località vicine più indicative perchè meglio conosciute nelle caratteristiche ambientali presso i quali si trova il luogo di raccolta, la data, e l'altitudine, desunta per lo più dall' altimetro [2].

Si spera che dette notizie, pur così povere e schematiche, riportate caso per caso, possano sempre dare a chi la desideri, una qualche idea più precisa sui micromiceti elencati nel presente contributo, e in particolar modo sui patogeni, i cui caratteri biologici e morfologici, così legati alla natura delle matrici, non sono perciò meno in rapporto direttamente o indirettamente con la natura dei luoghi [3].

Ringrazio il Prof. ARMANDO MAUGINI e il Dott. ANTONIO DE BENEDICTIS per avermi affidato questi studi e per l'assistenza datami in questa prima permanenza in Africa.

Ringrazio poi il Prof. CESARE SIBILIA, direttore dell' Istituto di Patologia vegetale dell' Università di Firenze, per la larga ospitalità concessami nel suo laboratorio, durante l'elaborazione di questo lavoro; il Prof. RAFFAELE CIFERRI, direttore dell' Istituto di Botanica agraria della stessa Università, per avermi permesso la consultazione della micoteca e della biblioteca private; e il Prof. ETTORE CASTELLANI, dell' Istituto agronomico per l'Africa Italiana, per i sussidi bibliografici fornitimi con amichevole sollecitudine.

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[1] Nei mesi di ottobre, novembre, dicembre e gennaio, nei quali la *P. viticola* è stata raccolta, ad Addis Abeba la temperatura in C° presenta rispettivamente i valori seguenti: t.media mensile = 16,2 ; 14,9 ; 14,6 ; 15,4; t.max. assoluta = 33 : 27,5 : 28 : 28; t.min. assoluta = 4 : 1,5 : 0,0 : 3. La pioggia (media trentennale) è rispettivamente di mm. : 22,7; 15,8; 11,1 : 11,3. L'umidità relativa di : 48 ; 45 ; 53 ; 48. [Da A. Fantoli in Agr. Col., vol. XXXI, pag. 500 (1937)].

[2] Talora l'altitudine indicata per i luoghi di raccolta non coincide con quella comunemente nota per le località designate. I dati da me riportati difatti si riferiscono particolarmente al punto dove si è eseguita la raccolta stessa; punto che per altitudine differiva a volte sensibilmente dalla località vicina di cui si è segnato il nome.

[3] Gli exsiccata delle specie elencate nel presente contributo sono conservati tutti presso la micoteca del Prof. Raffaele Ciferri, Direttore dell'Istituto Botanico di Pavia ed in parte presso il Laboratorio di Patologia vegetale dell'Istituto agronomico per l'Africa Italiana, Firenze.

E' per me gradito dovere ringraziare infine nel modo più vivo il Prof. Rodolfo Pichi-Sermolli, dell' Università di Firenze, al quale si debbono la determinazione delle matrici e le notizie sistematiche ad esse riferentisi.

Plasmopara viticola (Berk. e Curt.) Berl. e De Toni in Sacc., Syll. fungor., vol. VII, pag. 239. Su foglie di Vitis vinifera L., Mohamed-Aly (Addis Abeba), circa 2350 m.s.m., 28/10/1938 e 4/1/1939.

Taphrina deformans (Berk.) Tul. in Ann. Sc. nat., V Ser., vol. V, pag. 128 (1866); sub *Exoascus deformans* (Berk.) Fuck. in Symbol. mycol., pag. 252 (1869—70); Sacc., Syll. fungor., vol. VIII, pag. 816. Su foglie di Prunus persica Stokes coltivato, Bunno (Galla e Sidama), ca. 1700 m.s.m., 12/11/1938.

Meliola oleicola Doidge in Bothalia, vol. I, pag. 73 (1922)
var. **Jasmini** nov. var. (3111—4232) [1].

A typo differt: hyphopodiis capitatis cellula apicali plerumque irregulariter sub-clavata; hyphopodiis mucronatis interdum inter hyphopodia capitata dispersis; setis micelialibus μ 220—400 longis, peritheciis μ 160—220 diam.; sporidiis valde constrictis, μ 45—48 ⩔ 16—18.

Hab. in foliis Jasmini abyssinici R.Br. (Oleaceae), Ciora (Galla et Sidama) ca. 1900 m.s.m., 11/12/1938.

Il micete osservato forma plagule anfigene orbicolari di circa 2—4 mm., di diametro, spesso confluenti. Esso ha micelio olivaceo con cellule di μ 25—32 ⩔ 8—10, e ramificazioni opposte ad angolo acuto di circa 45°. Gli ifopodi capitati sono alternati, talora unilaterali, con cellula apicale irregolarmente angolosa, raramente sub-cilindrica (μ 8—12 ⩔ 15—20); e gli ifopodi mucronati, ampullacei, di solito opposti e siti su rami separati, talora però frammisti senza ordine a quelli capitati. Le sete, numerose specie intorno ai periteci, sono lunghe μ 220—400, larghe alla base μ 8—10 e sono diritte o leggermente arcuate, acute. I periteci infine, atri, globosi, misurano μ 160—220; e le spore, 4-settate, costrette, cilindracee, arrotondate alle estremità, μ 45—48 ⩔ 16—18.

Tra le numerose *Meliolae* segnalate su *Oleaceae*, alcune si avvicinano particolarmente al micete osservato. *M. busongensis* Hansford (3112—4222) [in Linnean Soc. Journ. Bot., Vol. LI, pag. 539 (1938)] però ne differisce nettamente per gli ifopodii capitati opposti; e *M. Tayabensis* H.S. Yates (3111—4221) [in Philippine Journ. Science, vol. XII, pag. 369 (1917)] per le plagule ipofille di cm. 1—2, e perchè ha ifopodi capitati globosi, sete più corte (250—300) acute o ottuse, peritecii e sporidii minori.

La perisporiacea in esame sembra invece più vicina a *M. oleicola* Doidge (3111—4331) [2] (loc. cit.) come varietà nuova. Dal tipo, il micete osservato, raccolto in località assai diversa e su altra matrice, differisce morfologicamente per i caratteri della diagnosi.

Corynelia uberata Fries in Syst. mycol., vol. II, pag. 535 (1822); sub. *C. clavata* (L.) Sacc. in R. Pirotta, N. Giorn. Bot. It., Vol. XXI, pag. 313 (1889); Sacc., Syll. fungor., vol. IX, pag. 1073. Su foglie di Podocarpus gracilior Pilger [= P. falcata Engler, non (Thunb.) R. Br. ex Milb.], presso Bedelle, 6/12/1938, Soddu, 25/1/39 (Galla e, Sidama), e Shambo, 10/1/1939 (Scioa), ad altitudini comprese tra 1700—2100 m.s.m.

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[1] Successivamente questa varietà è stata ridescritta da E. Castellani come: *Meliola jasmini* Hansf. et Stev. var. *major* [(E. Castellani, N. G. Bot. Italiano, vol. XLIX, pagg. 4—5 (1942)].

[2] In Doidge e H. Sydow: The South African species of the Meliolineae (Bothalia, vol. II, parte 2 pag. 458) a questo fungillo segue la formula 3111.4332; ma esso ha spine di μ 240—300.

Il riferimento di *Corynelia clavata* a *C. uberata* si deve a Fitzpatrick [Mycol., vol. XII, pag. 247 (1920)]. *P. falcata* Engler, non (Thunb.) R. Br. ex Milb., su cui si ha la segnalazione di Pirotta (loc. cit.) della *C. clavata* (L.) Sacc.,. è stata invece riportata a *Podocarpus gracilior* dopo esame del materiale originale conservato nell' erbario coloniale dell' Istituto Botanico di Firenze. Quest' ultimo riferimento del resto concorda con le conclusioni generali sui *Podocarpus* etiopici di R. Pilger [in Engler, das Pflanzenreich, 18 Heft (IV, 5), pag. 71—72]. Nè la precisazione sembra priva di interesse, poichè si suppone (Fitzpatrick, loc. cit.) che tra le diverse *Coryneliae* e le varie specie di *Podocarpus* esistano stretti rapporti biologici di specializzazione.

Uncinula Sibiliae n.sp.

Epiphylla; micelio sub-persistente, albido, tenui, effuso; peritheciis in eo insidentibus punctiformibus, plerumque sparsis, interdum hic inde gregariis; primo flavidis, globosis, dein umbrinis vel atrobrunneis, depressis vel leniter inflexis, *μ* 150—170 diam., contextu membranaceo irregulariter (pseudo-) celluloso, *μ* 10—20 ⩔ 8—14; appendicibus numerosis, 50—55, perithecii diametrum sub-aequantibus, *μ* 120—170 long., hyalinis, rectis vel curvulis, simplicibus, ad basim I-septatis, ibique ca. *μ* 4 crassis, quandoque asperulis, sursum non vel leniter inflatis, apice uncinatis, vel arcte circinatis; ascis usque 10—12 in quoque perithecio, sub-globosis vel late obovatis, brevissime crasseque pedicellatis, *μ* 58—62 ⩔ 37—40; sporidiis plerumque 4—6, chlorinis, ovoideis, utrinque rotundatis, *μ* 12—16 ⩔ 18—22.

Hab. in foliis Stereospermi Kunthiani Cham. (Bignoniaceae), prope Jaio (Galla et Sidama) ca. 1700 m.s.m. 8/12/1948.

Il parassita forma, sulla pagina superiore fogliare, macchie appena polverulente, non sollevate, bianchissime, a contorno grossolanamente circolare, le quali confluiscono assai spesso occupando in gran parte o del tutto l'epifillo delle foglie attaccate. La forma ascofora, raccolta due mesi dopo il termine delle grandi piogge a Jaio sulla pista di Gore, si presenta per lo più immatura. I primi periteci sembrano originarsi alla periferia delle macchie bianche, punteggiandone in seguito la superficie, ed appaiono talora disposti quasi concentricamente, più di frequente sparsi senza regolarità, di rado distintamente gregari. I periteci membranacei, di *μ* 150—170, visibili inizialmente come minuscoli punti globosi giallo-aranciati, a maturità divengono depressi, talvolta leggermente infossati superiormente, ed hanno intensa colorazione bruna. I fulcri si originano sempre in posizione equatoriale, sono di solito alquanto rigidi e, allungandosi, si intrecciano tra loro; cosicchè può accadere di sollevare per uno solo diversi corpi fruttiferi tra loro connessi. Detti fulcri, ialini, diritti, di solito 1-settati alla base e talora anche 2-settati, presentano in qualche caso sulle pareti minuscoli nodi o gibbosità intensamente rifrangenti simili a quelli notati in *Uncinula salicis* (D.C.) Wint. da J. Francais [Rev. de Pat. végét. et d'entom. Agr., vol. XX, pag. 267 (1933)] [1]); essi hanno estremità superiore uncinata o strettamente ravvolta su sè stessa, talora leggermente rigonfiata. I fulcri, in numero di 50—55 per ogni peritecio, misurano *μ* 120—170 ⩔ 4 (alla base). Negli aschi globosi, quasi sessili, assai ricchi di grosse gocciole oleose giallognole, si costituiscono tardivamente quattro-sei ascospore ovoidi, molto arrotondate, clorine.

Il non aver ritrovato nella letteratura precedente segnalazioni di *Uncinulae* su l'intera famiglia delle *Bignoniaceae*, prevalentemente distribuita in regioni tropicali o sub-tropicali, e l'essere indubbiamente anche in queste forme

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[1]) Giustamente J. FRANÇAIS nota che la presenza di queste ,,ampules hémisphériques" non ha alcun rapporto con l'età del fulcro.

(Erysiphaceae), seppure in vario grado, la natura del parassita in stretto rapporto con quella degli ospiti, hanno indotto a ritenere nuovo il micete raccolto e come tale a descriverlo.

Eccettuate alcune forme di *Ovulariopsis* [*O. obclavata* Wakef. e *Phyllactinia suffulta* Sacc. var. *rigida* Salmon (stato conidico)] 2) si ha notizia di un solo *Oidium* (*O. bignoniae* Jaczewski) segnalato su *Bignoniaceae (B. catalpa)*, in rapporto con *Erysiphe communis* Grév. f. *Bignoniae* Jaczewski. Lo stato conidico dell' *Uncinula* ora descritta, che non è stato possibile osservare nel materiale esaminato, rimane pertanto indeterminato.

La specie è stata dedicata al Prof. CESARE SIBILIA, direttore dell' Istituto di Patologia Vegetale dell' Università di Firenze.

Gnomonia Grewiae n.sp.

Maculis amphigenis, primo subrotundatis dein plus minusve irregularibus, confluentibus, magnam foliorum partem occupantibus, in epiphyllo griseo-testaceis, centro pallidioribus, late fusco-marginatis, in hypophyllo flavido-viridulis; peritheciis hypophyllis, sed in utraque foliorum pagina conspicuis et leniter prominentibus, in sicco collapsis, sparsis vel gregariis, globosis, olivaceis, membranaceis, *μ* 170—200 diam., contextu pseudo-celluloso, irregulariter polygonio *μ* 8—12 ⩔ 8—10, rostello cylindraceo, *μ* 80—130 ⩔ 16—30, infra dilute olivaceo, superne melleo, fere albido, tenuissimo, intus periphysibus dense vestito, in apice vel prope apicem lateraliter dehiscente; ascis numerosis aparaphysatis, 8-sporis, subfusiformibus, *μ* 45—55 ⩔ 10—12, breve pedicellatis; sporidiis oblique distichis, fusoideo falcatis, gibbosis, plerumque ad basim I-septatis, 1—2 guttulatis, sub-hyalinis vel chlorinis, *μ* 11—13 ⩔ 3—4.

Hab. in foliis Grewiae ferrugineae Hochst. et Grewiae spp. (Tiliaceae), Jambo, prope Gore, 8/12/1938, et Gimma, 19/11/1938, (Galla et Sidama), ca. 1700—1900 m.s.m.

Su foglie di Grewia spp. si osservano spesso nella pagina superiore macchie tra il laterizio e l'umbrino, più chiare al centro, circondate da un alone scuro gradualmente sfumante all' esterno, in corrispondenza delle quali inferiormente il lembo fogliare presenta diffuse decolorazioni giallo-verdastre. Le aree interessate si mostrano densamente punteggiate di nero e più visibilmente sulla pagina superiore glabra. Inferiormente, a forte ingrandimento, si osservano al centro dei sopraddetti puntolini neri — i peritecii del parassita —, i rostri di questo, in forma di minuscoli cilindretti eretti di tonalità mellea, assai fragili, che, perforando l'epidermide, sporgono all' esterno. I periteci del micete, di *μ* 170—200, globosi, olivacei, membranacei, a struttura pseudo-parenchimatica ben distinta, superano in diametro lo spessore della foglia, emergendo così su ambedue le facce di questa, e contengono numerosissimi aschi brevemente pedicellati, attenuati ad ambedue le estremità, non parafisati, ottospori, misuranti *μ* 45—55 ⩔ 10—12. Le ascospore, di *μ* 11—13 ⩔ 3—4, jaline, sono sottili, fusoideo-falcate, talora in prossimità di un apice gibbose e spessissimo, specie in questo ultimo caso, divise in due cellule ineguali, delle quali la più bassa è anche nettamente più piccola (*Apiognomonia* v. Hoen.) Alla sommità dei periteci, in corrispondenza dell' ipofillo, si pronunzia poi il rostro olivaceo, densamente perifisato, largo *μ* 22—26, arrotondate all' apice, che, sollevata l'epidermide, la perfora, rimanendone però talora strettamente cinto, sicchè il suo diametro può a quest' altezza ridursi a *μ* 16—18. Questo rostro centrale è olivaceo alla base e per un' altezza di circa *μ* 30—35 fino a che cioè rimane coperto dall' epidermide sollevata; superata questa, si accresce

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2) Questa varietà è poi stata da Blumer distinta come specie (Beitr. zur Krypt. flora der Schweiz, VII, 1, (1923)).

rapidamente, se così può supporsi dalla rarità degli stadii intermedi di sviluppo, in forma di cilindro melleo, eretto, fragile, talora leggermente più crasso all' apice, fino a μ 75—130 di lunghezza.

La parete del rostro, superiormente alla porzione basale di colorazione olivacea e disegno pseudo-cellulare ben distinto, si assottiglia in brevissimo spazio, assumendo tonalità assai chiara e spessore minimo, difficilmente valutabile.

Le lunghe perifisi, che si originano al di sopra dello strato proligero e alla base del rostro, si spingono attraverso lo stretto collo nella porzione chiarissima di questo, che ne è piena, e convergono gradualmente con l'estremità libera verso il canale centrale da esse delimitato. Il rostro si apre all' apice o si lacera lateralmente in prossimità di questo. Non è parso troppo ardito supporre che allo svilippo rapidissimo della parte libera contribuiscano le perifisi in accrescimento, le quali ne distenderebbero la sottile parete e, talora forse con inizi di gelificazione, ne rigonfierebbero l'estremità, che come si è detto può lacerarsi lateralmente.

1) *Sphaerella Gardeniae* Cooke in Journ. of Bot., Vol. IX, pag. 108 (1883); Sacc., Syll. fungor., vol IX, pag. 642. Su foglie di Gardenia lutea Fresen, pressi del Nilo Azzurro (alle bocche del Lago Tana), ca. 1750 m.s.m., 26/2/1939; socia di Phyllosticta gardeniae F. Tassi.

Secondo la diagnosi assai breve di Cooke (loc. cit.) il micete, riscontrato su foglie di *Gardenia florida* L. (= *G. jasminoides* Ellis), avrebbe peritecii epifilli e non ipofilli, come nell' esemplare esaminato. La concordanza degli altri caratteri della descrizione, la presenza nelle macche della *Phyllosticta gardeniae* F. Tassi, probabilmente identica a *P. gardeniae* Cooke (vedi in seguito), inducono ciò nonostante a riferire a detta specie l'esemplare etiopico.

Sphaerella sp.

Maculis amphigenis castaneo-ferrugineis, sub-orbicularibus vel irregularibus, zonatis dein fissis, interdum nervis limitatis, fusco-elevato-marginatis, in hypophyllo laetioribus; peritheciis amphigenis, olivaceis, immersis, globosis, μ 90—100 diam., ostiolo papillato vix prominulo, μ 10—15 lato, erumpentibus, contextu membranaceo pseudo-celluloso, μ 8—10 ⩔ 8—12; ascis clavato-elongatis, rectis vel leniter curvatis, subsessilibus, 8-sporis, μ 42—52 ⩔ 10—14, aparaphysatis; sporidiis I-septatis, hyalinis, constrictis, utrinque attenuatis, subdistichis, loculo inferiore angustiore, μ 14—16 ⩔ 8—10.

Hab. in foliis Manihotis Glaziovii Muell. (Euphorbiaceae), Saddu, prope amnem Baro (Galla et Sidama), ca. 1300 m.s.m., 15/12/1938.

Le macchie fogliari anfigene hanno colorazione fondamentalmente castanea, sfumante al grigiastro inferiormente, più intensa nella pagina superiore, e sono assai distintamente zonate di scuro su ambedue le faccie del lembo fogliare. Tendenzialmente orbicolari in principio, assumono poi forma talora assai varia e in ultimo si fendono irregolarmente. Esse hanno margine più scuro nettamente sollevato.

Per i caratteri macroscopici pertanto la presente alterazione è diversa da quelle di *M. utilissima* Pohl riferibili al parassitismo di *Cercospora Henningsii* Allesch. e *C. carybaea* Chupp e Cif. delle quali l'ultima sarebbe in

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1) Per la sinonimia di questo genere si è creduto opportuno attenersi alle ,,International rules of botanical nomenclature" (1935), ove a pag. 122 si propone di eliminare *Mycosphaerella* Johans. a favore di *Sphaerella* Ces. et De Not., per accordi intervenuti con la Commissione algologica. A questa proposta si è più recentemente aggiunta la raccomandazione della ,,Nomenclature Committee of the British Mycological Society" [in Trans. Brit. Mycol. Soc. pagg. 220—22 (1939)] di preferire *Sphaerella* (Fr.) Rabenh. a *Sphaerella* Ces. et De Not., *Sphaerella* Sommerf., *Carlia* Rabenh., *Mycosphaerella* Johans.

relazione con *Mycosphaerella manihotis* Syd. o *M. manihotis* Ghesquière e la seconda probabilmente sarebbe in rapporto con una *Mycosphaerella* sp. trovata da Ciferri e da Solheim su ,,exsiccata'' di S. Domingo [1]). I peritecii, anfigeni, immersi, regolarmente globosi, affiorano alla superficie per mezzo dell' ostiolo (*μ* 10—15) papillato, appena sporgente all' esterno. Essi sono membranacei, a contesto pseudo-parenchimatico irregolarmento poligonale, di *μ* 8—12 ⩔ 8—10. Gli aschi, non parafisati, di *μ* 42—52 ⩔ 10—14, sono allungati, sub-cilindrici, leggermente clavati, quasi sessili; e contengono 8 ascospore ialine, più o meno regolarmente distiche, diritte o leggermente curvule, gradualmente assottigliate alle estremità, bicellulari. La cellula superiore è alquanto più larga dell' altra e in prossimità del setto si restringe più o meno sensibilmente.

Il micete osservato, pur concordando in genere per caratteri morfologici con *Mycosphaerella manihotis* Sydow — secondo la descrizione data in Bull. de l'Herb. Boiss. pag. 78 (1901) — ne differisce però sensibilmente per dimensioni [peritecii *μ* 65—85 diam.; aschi *μ* 27—35 ⩔ 5—8; ascospore (non costrette) *μ* 10—12 ⩔ 1,5—2,5]. E, le differenze sopra dette non sembrando trascurabili, si sarebbe indotti a ritenere distinta dalla prima la forma osservata su *Manihot Glaziovii*

Tuttavia, nell' impossibilità di poter consultare il più recente lavoro di Ghesquière [in Bull. Inst. Roy. Col. Belge, vol. III, pag. 160—178 (1932)] su *M. manihotis* Ghesquière et Heur. e di poter confrontare il micete osservato con la forma ritrovata su ,,exsiccata'' di *C. caribaea*, si è ritenuto più prudente descrivere per ora la forma ascofora raccolta come *Sphaerella* sp. e di proseguire intanto per qualche tempo le osservazioni su di essa.

Leptosphaeria Michotii (Westd.) Sacc. in Fungi Ital., Tav. 279 (1878); Sacc., Syll. fungor., vol. II, pag. 58. Su brattee fiorali di Cyperus papirus L., rive del lago Tana, ca. 1850 m.s.m., 22/2/39.

Di questa *Eu-Leptosphaeria* le dimensioni delle ascospore bisettate (*μ* 18—24 ⩔ 5) sono nettamente superiori a quelle del tipo (*μ* 16—18 ⩔ 3,5—4,5).

Nell' esemplare esaminato inoltre l'episporio non è costretto ad ambedue i setti (,,*sporidiis ad septa leniter constrictis*''), ma ad un solo. Essendo però, come è noto, questo carattere morfologico abbastanza comune nelle specie del complesso genere tipicamente sferiacee, ravvicinate da v. Hoehnel [Ber. der. Deut. Bot. Ges., vol. XXXVI, pagg. 135—140 (1918)] a *Nodulisphaeria* Rabh., si è piuttosto indotti a pensare ad imprecisione della diagnosi originale.

Micronectria Sizigii n.sp.

Peritheciis epiphyllis, epidermide bullata rufescente tectis, in greges usque 1 cm. diam. dense confertis, purpureo-cinctis, plerumque globosis, regularibus, ca. *μ* 300—450 diam., levibus, periphysatis, ostiolo conoideo-truncato matricem perforante quandoque vix prominulo, contextu olivaceo-fusco parum distincto, infra membranaceo, sursum sensim densiore, crassiore, circa ostiolum *μ* 35—40 lat.; ascis cylindraceis, curvatis, breve stipitatis, octosporis, ca. *μ* 145—155 ⩔ 14—16; paraphysibus non visis; sporidiis filiformibus, utrimque obtuse rotundatis, eseptatis, guttulatis, chlorinis, *μ* 135—145 ⩔ 3.

Hab. in foliis vivis Syzigii guineensis D.C. (Myrtaceae), Uoccià, ad fluminem Diddessa, ca. 1650 m.s.m., 3/12/1938; prope Omo-Bottego, ca. 1600 m.s.m., 25/1/1939; Gondar, ca. 2000 m.s.m., 27/2/1939; Asosa, ca. 1500 m.s.m., 1/4/1932, socia Scolescosporii syzigii n.sp.

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[1]) Queste e le seguenti notizie relative ai rapporti metagenetici tra *Cercospora* e *Sphaerella* mi sono state gentilmente fornite dal Prof. R. CIFERRI, che di nuovo ringrazio.

Le foglie di Syzigium nelle quali è presente questa *Micronectria, mostrano* qua e là nel lembo, sulla pagina superiore, in particolare tra le nervature secondarie parallele (le foglie sono penninervie), macchie oblunghe di tonalità purpureo-vinosa a superficie sollevata e densamente punteggiata di minuscole convessità più scure. In corrispondenza di queste macchie inferiormente il lembo fogliare si presenta prima diffusamente violaceo, poi gialliccio e secco.

I peritecii, di solito regolarmente globosi, sono densamente gregari nelle macchie rossastre, nelle quali, come si è accennato, si rivelano come minute prominenze più scure, velate dall' epidermide. Essi sono immersi nel mesofillo e circondati da una parete olivacea, membranosa, a struttura indistinta, che superiortmente diviene più scura e spessa, quasi carbonacea, raggiungendo intorno all' ostiolo μ 40 e più; l'ostiolo è tronco-conico, ma rimane sempre coperto dall' epidermide sollevata, meno che nel poro appena sporgente. Internamente al peritecio non si sono osservate parafisi; al di sopra dello strato proligero invece si differenziano numerose perifisi flessuose, filiformi, che, seguendo l'andamento dell' excipulo fino alla sommità, ricadono poi a riempire la cavità del peritecio nello spazio soprastante gli aschi. Questi sono lungamente cilindracei, brevemente pedicellati, 8-spori, curvi verso il centro del nucleo. Gli sporidii, leggermente più corti degli aschi, da giovani sono spesso ravvolti ripetutamente a spirale, come in *Cochliobolus* Drechsl., ma a maturità si presentano in modo vario, talora distesi o sinuosi, tal' altra più o meno lassamente spiralati; essi sono multiguttulati, non settati e all' estremità brevemente arrotondati.

Il micete osservato, che in un primo tempo era sembrato ravvicinabile ad *Ophiobolus porphyrogonus* (Tode) Sacc., parve poi con sicurezza attribuibile, pel suo ,,habitus'' generale, a *Micronectria* tra le *Hypocreaceae.* Nel genere il fungo in esame è assai simile a *M. Eugeniae* Petch [T. Petch, Hypocr. zeyl. in Ann. R. Bot. Gard. Peradeniya, vol. VII, pag. 137 (1920)], da cui si distingue tuttavia, non tanto per la matrice (*Eugenia* sp.), quanto per la colorazione assunta da questa, la posizione ipofilla dei periteci, le dimensioni di essi, la forma e le misure delle ascopore ,,*obscure*'' settate.

Il fungo del *Syzigium* si trova quasi sempre associato ad un melanconiale: *Scolecosporium syzigii* (descritto in seguito), il quale occupa specialmente in margine e sulla pagina inferiore le aree decolorate nelle quali la *Micronectria* è presente e talora si mostra quasi sovrapposto ai corpi fruttiferi dell' ascomicete. Si possono però raramente osservare acervuli di *Scolecosporium* in assenza di *Micronectria* o più difficilmente i periteci di quest' ultima da soli.

D'altra parte non si ha notizia di rapporti metagenetici fra *Micronectria* e *Scolecosporium* e la sola forma ascofora in supposto rapporto con *Scolecosporium* (*S. fagi* Lib.) sarebbe una *Massaria*: *M. macrosperma* [1]).

Phyllachora Bersamae Lingelsh. in Engl. Bot. Jahrbuch., vol. XXXIX, pag. 604 (1907); Sacc., Syll. fungor., vol. XXIV, pag. 598; sub *Physalospora bersamae* Syd. in Deutsche Zentr. Afr. Exped., II, pag. 98 (1907—1908); Sacc., Syll. fungor., vol. XXII, pag. 82. Su foglie di Bersama abyssinica Fres., rive del fiume Gogieb, 21/11/1938, valle del fiume Omo vicino Soddu, 25/1/1939 (Galla e Sidama), pressi di Addis Abeba, 12/1/1939, ad altitudini comprese tra circa 1600—2300 m.s.m.

Questo fungillo è singolare per la presenza di uno stroma a clipeo anomalo, quasi nullo, di particolari colorazione e struttura (*Plectosphaeria* Theiss.) Si deve a Petrak [Ann. Mycol., vol. XXXII, pag. 413 (1934)] il suo definitivo riferimento a *Phyllachora bersamae.*

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[1]) Di questa forma ascofora riportata in Sacc., Syll. fungor., vol. III, pag. 782, sotto *Scolecosporium fagi,* non si trova riscontro in altre parti della Sylloge stessa; nè sembra identificabile con *M. macrospora* (Desm) Sacc. (vol. II, pag. 10) o con *M. macrospora* B. e C. (vol II, pag. 133, sub *Aglaospora profusa* Not.).

Phyllachora Cynodontis (Sacc.) Niessl in Not. Pyr., pag. 54; Sacc., Syll. fungor., vol. II, pag. 602. Su foglie di Cynodon sp., Mohamed Aly (Addis Abeba), ca. 2400 m.s.m., 29/10/1938.

Phyllachora Eleusines Speg. in Myc. arg., Ser. II, pag. 75 (1902); Sacc., Syll. fungor, vol. XVII, pag. 839; sub *Phyllachora eleusines* P. Henn. in Ann. Mus. du Congo, vol. II, pag. 98 (1907); Sacc., Syll. fungor., vol. XXII, pag. 426. Su foglie di Eleusine coracana Gaertn., Jambo, presso Gore (Galla e Sidama), ca. 1800 m.s.m., 9/12/38.

La segnalazione di P. Hennings è stata riferita alla specie di Spegazzini da Theissen e Sydow [Ann. Mycol., vol. XIII, pag. 448 (1915)].

Phyllachora Gondarensis n.sp.

Stromatibus sub-cuticularibus, epiphyllis, in macula indeterminata primo flavida dein ferruginea insidentibus, hemisphaerico-prominulis, rotundatis, nitidis, atris, punctiformibus, sparsis, quandoque sub-confluentibus, μ150—250 diam., typice I-locularibus, loculis minute ostiolatis, epidermide leniter infossis, μ 130—220 ⩔ 70—100 diam.; ascis late obovato-clavatis, sub-sessilibus, 8-sporis, μ 56—62 ⩔ 20—24; sporidiis irregulariter dispositis, continuis, ellipsoideis, uno fine rotundiore, hyalinis, intus granulosis, μ 20—24 ⩔ 10—12.

Hab. in foliis Stephaniae abyssinicae Walp. (Menispermaceae), Bar-dar (Amara), ca. 1850 m.s.m., 25/1/1939.

Gli stromi di questo micete sono visibili come puntolini neri solo sulla pagina superiore fogliare in *Stephania abyssinica.* In corrispondenza di essi, dopo qualche tempo, il tessuto fogliare assume chiara tonalità gialla, e poi, a cominciare dal centro, ocraceo-ferruginea.

Gli stromi, di circa 1/5 di mm., uniloculari, emisferico-depressi, duri, lucenti, minutamente ostiolati, talora aggregati (da 2 a 4), si fendono largamente a maturità. Nei loculi (μ 130—220 ⩔ 70—100) si osservano aschi parafisati, quasi sessili, ovoidi, misuranti ca. μ 56—62 ⩔ 20—24, e contenenti senza ordine ascospore (8) ialine, continue, elissoidali, arrotondate a un' estremità, più attenuate all' altra, di μ 20—24 ⩔ 12—20.

Per la posizione degli stromi, chiaramente sub-cuticolare, e per gli altri caratteri morfologici questo fungillo andrebbe riferito al gen. *Trabutia* Sacc. e Roum. [emend. Petrak in Ann. Mycol., vol. XXVIII, pag. 386 (1929)]. Si è preferito però di attribuirlo, benchè il caso presente non sembri dubbio, al gen. *Phyllachora,* seguendo l'indirizzo che tende a semplificare la sistematica di questo gruppo di *Dothideales,* per la moltitudine delle forme di passaggio (Clements e Shear, The genera of Fungi, 1931; E. Castellani e Ciferri, Prodromus Mycofl. Afr. Or. It., 1937).

Su *Stephania capitata* Spreng., a Giava, è stata già segnalata una *Trabutia* (*T. Stephaniae* Rac.), riportata da Theissen e Sydow [Ann. mycol. vol. XIII, pag. 475 (1915)] a *Phyllachora.* Questo micete però, per ,,habitus'', dimensioni, caratteri morfologici e posizione del corpo fruttifero nei tessuti matricali, è senza dubbio diverso da quello raccolto presso il lago Tana.

Phyllachora Lactea Theiss. e Syd. in Ann. mycol., vol. XIII, pagina 497 (1915); sub *Auerswaldia dalbergiae* P. Henn. in Engl. Bot. Jahrbuch, vol. XXXVIII, pag. 114 (1905); Sacc., Syll. fungor., vol. XXII, pag. 428. Su foglie di Dalbergia sp., presso il fiume Omo Bottego nelle vicinanze di Soddu (Galla e Sidama), ca. 1700 m.s.m., 25/1/1939.

Questa specie e *Phyllachora clemensiae* Petr. [in Ann. mycol., vol. XXIX, pag. 208 (1931)] sono vicinissime, quasi varietà o forme di *Phyllachora spissa* Syd. [in Ann. mycol., vol IX, pag. 397 (1911)]. Il micete osservato, tuttavia, concorda pienamente con *P. lactea* per i pochi caratteri dimensionali utili per questo riferimento specifico.

Endodothella Albizziae (Syd.) Syd. in Ann. mycol., vol. XIII, pag. 584 (1915); Sacc., Syll. fungor, vol. XXIV, pag. 614; sub *Dothidella albizziae* Syd. in Philippine Journ. of Sci., vol. VIII, pag. 280 (1913). Su foglie di Albizzia Schimperiana Oliv., Colitu, presso Soddu (Galla e Sidama), ca. 1700 m.s.m., 29/1/1939.

L'esemplare osservato presenta misure alquanto maggiori del tipo negli aschi (*µ* 70—85 ⩔ 15—16) e nelle ascospore (*µ* 14—17 ⩔ 7—8). Se ne è poi conservato il riferimento al gen. *Endotothella* Theiss. e Syd. (= *Dothidella* Speg. p.p.), benchè più recentemente [Clements e Shear, The genera of fungi, pag. 294 (1931)] detto genere sia stato messo in sinonimia con *Placostroma* Theiss. e Syd.

Hysterographium Graminis Ell. e Ev. in Bull. Torrey Bot. Club, vol. XXVII, pag. 53 (1900); Sacc., Syll. fungor., vol. XVI, pag. 668. Su culmi secchi di Hyparrhenia sp., presso Gondar (Amara), ca. 1950 m.s.m., 2/3/1939.

Melampsora Lini (Pers.) Lév. in Ann. Scienc. Nat., III, vol. VIII, pag. 376 (1847); P. e. H. Sydow, Monogr. Uredin., vol. III, pag. 381. Su foglie di Linum usitatissimum L. coltivato, Acachi (Scioa), ca. 2100 m.s.m., 3/11/1938.

Melampsora Ricini (Biv.) Pass. in Erb., critt. ital., II, n. 684; P. e H. Sydow, Monogr. Uredin., vol. III, pag. 391. Su foglie di Ricinus communis L. coltivato, Oletta (Scioa), ca. 2400 m.s.m., 31/12/1938.

Phakopsora Erythraea Sacc. in Ann. Mycol., vol. VIII, pag. 336 (1910); P. e H. Sydow, Monogr. Uredin., vol. II, pag. 416. Su foglie di Stereospermum Kunthianum Cham., Saddu, nella valle del Baro (Galla e Sidama), ca. 1300 m.s.m., 15/12/1938.

Nella forma teleutosporica si sono riscontrati anche, sebbene più di rado, sori epifilli.

Nella pagina inferiore poi, frammisti ad essi, si sono osservati numerosi uredosori, puntiformi, cinnamomei, parafisati, con spore globose o sub-trigone, echinulate, castaneo-scure, di *µ* 18—20 ⩔ 22—25, e con pori di germinazione ben distinti. Tale forma corrisponde a quella osservata dai Sydow (loc. cit.) su foglie di *Stereospermum kunthianum* var. *dentatum* A. Rich. affette da *Phakopsora erythreae* e da questi autori riferita con riserva alla forma teleutosporica sopra detta. Il riferimento sembra così confermato.

Physopella Fici (Cast.) Arthur in Rés. Sci. Congr. Bot. Vienna, pag. 338 (1906); sub *Kuehneola fici* (Cast.) Butl. in P. e H. Sydow, Monogr. Uredin., vol. III, pag. 323. Su foglie di Ficus vallis Choudae Delile, presso Adamitullo (Galla e Sidama), ca. 1700 m.s.m., 31/1/1939.

Questa ruggine è stata osservata solamente nella forma uredosporica (*Uredo fici* Cast. var. *abyssinica* P. Henn.).

Ravenelia Baumiana P. Henn. in Bot. Ergebn. der Kunene-Sambesi Exped., pag. 157 (1902); P. e H. Sydow, Monogr. Uredin., vol. III, pag. 262. Su foglie di Cassia goratensis Fres., Zeghiè sul lago Tana, ca. 1850 m.s.m., 23/2/1939.

Le papille cilindriche, apicalmente dilatate, di circa *µ* 8—11 ⩔ 4—5, facilitano il riconoscimento di questa specie.

Ravenelia Candussioi n.sp.

Uredosoris amphigenis et petiolicolis, sparsis, rotundatis, epidermide fissa cinctis, pulveraceis, ferrugineis, applanatis, μ 350—380 diam., paraphysibus cylindraceo-clavatis plus minusve gracilibus, μ 2—10 latis, usque μ 60—70 longis; uredosporis ovatis laxe echinulatis, dilute aurantiacis, infra pallidioribus, poris germ. 4 sub-aequatorialibus praeditis, μ 14—17 ⩗ 10—14; teleutosoris amphigenis et petiolicolis, sparsis vel gregariis, saepe late confluentibus, mox erumpentibus, atris, rotundatis vel irregularibus, usque 1,5—2 mm. diam.; capitulis teleutosporarum castaneis, ambitu sub-orbicularibus, μ 80—110 diam., convexis, infra inflexis, e sporis 5—8 in omni directione compositis, quaque spora papillis 4—5 hyalinis, conicis, ad basim ca. μ 2—3 latis, μ 4—5 longis, obsita; sporis singulis μ 24—34 altis, μ 14—22 latis, episporio ad apicem μ 4—5 crasso; cystidiis sub-globosis, adpressis, ut videtur eodem numero quo sporae singulae; pedicello deciduo, hyalino, composito.

Hab. in foliis Acaciae laetae R.Br., Adi-Ugri (Erythraea.), ca. 200 m.s.m., 20/1/1939. Leg. Dr. R. Candussio, a quo speciei nomen.

Si sono rinvenuti pochi uredosori anfigeni e peziolicoli, di forma regolarmente circolare e colorazione ferruginea piuttosto chiara. Le uredospore di pallida tonalità aranciata, hanno contorno ovale, sono alquanto espanse all' apice, e si inseriscono largamente sul pedicello bene sviluppato. Si sono osservati quattro pori di germinazione, spostati leggermente nella metà superiore.

Alle uredospore sono frammiste parafisi superanti talora la superficie libera del soro. Negli ,,exsiccata'' esaminati, queste parafisi a parete sottilissima si osservano rigonfiate a mo' di clava nelle porzioni periferiche del soro stesso, ma nell' interno di questo si mostrano di solito cilindracee e, forse anche perchè densamente stipate e per la pressione delle parti adiacenti in accrescimento, quasi svuotate, striminzite, gracilissime, talora quasi filiformi. Le loro dimensioni, che possono anche raggiungere in lunghezza μ 60—70, oscillano pertanto in spessore da μ 1—2 a μ 10 (negli elementi periferici ben rigonfi) 1). I teleutosori, abbondantissimi nell' esemplare esaminato, sono atri e largamente confluenti. I capitoli delle teleutospore, di intensa colorazione castanea, sono costituiti da un gran numero di spore, le periferiche abbastanza regolarmente disposte in due cerchi concentrici. Detti capitoli, convessi, inferiormente sensibilmente cavi, portano al disotto copiose cisti ialine, una per ogni spora; quelle esterne rigonfiate a fiasco, le interne densamente stipate e per reciproca pressione lateralmente poligonali. L'intero capitolo è poi rivestito di una membrana ialina, continua, di circa μ 2 di spessore, sulla quale sporgono, in numero di 4—5 per ogni cellula, papille coniche, semplici, non acuminate, ialine o clorine, lunghe μ 4—5 e larghe alla base μ 2—3. Le singole spore unicellulari, cuneiformi quelle interne, esternamente arrotondate le marginali, sono alte circa μ 24—34, larghe ca. μ 6—8 inferiormente e μ 14—22 superiormente, ed ivi hanno parete più intensamente colorata, ispessita (ca. μ 4). Il pedicello deciduo è composto di 4 elementi ialini.

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1) Ci si è soffermati alquanto sul modo di presentarsi delle parafisi negli uredosori della *Ravenelia* in esame, perchè su questo non sembra si abbiano esempi in altre specie del genere. E solo raramente l'osservazione di varie *Raveneliae* negli ,,exsiccata'' del prof. Ciferri ha permesso di notare qualche cosa di simile. In *R. monosticha* Speg. si trovano descritti [in Ann. Mus. Nac. Hist. nat. Buenos Aires, vol. XXXI, pag. 379 (1923)] sottili corpi bacillari, quasi fusariformi, di μ 28—32 ⩗ 3—4, frammisti alle uredospore. Queste formazioni pero non sembrano omologhe a parafisi.

2) In Etiopia sono state finora ritrovate su *Acaciae* solo: *R. Volkensii* P. Henn., cecidiogena, e *R. acaciae-melliferae* Bacc., a teleutospore lisce, da Sydow identificata con *R. Schweinfurthii* (in Ann. di Bot., vol. XIV, pag. 128 (1917)).

I caratteri morfologici delle uredospore e dei capitoli di teleutospore, larghi, schiacciati, inferiormente convessi, con teleutospore indivise e cisti in numero uguale al numero di queste, il modo di presentarsi delle parafisi nello interno degli uredosori di questa forma non caulicola, non coincidono con quelli delle specie di *Ravenelia* già segnalate sul genere *Acacia* 1) e riportate in Dietel, Ueber die Gattung *Ravenelia*, Beitr. z. Bot. C. Blatt (vol. XX, pagg. 343—411), P. e H. Sydow, Monogr. Uredin. (vol. III, pagg. 224—310), in Saccardo, Syll. fungor. (vol. XXIII, pagg. 793—797), e con quelle più recentemente descritte da Sydow negli Ann. mycol., vol. plur., da Arthur [Man. of. rusts. in U.S.A. and Canada, Lafayette, pagg. 63—67 (1934)] da Mundkür e Prasad [Mycol., vol. XXX, pagg. 685—688 (1938)].

Con particolare cura si è poi confrontata questa ruggine dell' *Acacia laeta* con le cinque specie di *Ravenelia* istituite da Doidge 1) nel Sud Africa [in Bothalia, vol. II, parte I, pag. 146 (1927) e vol. III, parte IV, pagg. 504—505 (1939)], e cioè con: *R. peglerae* su foglie e legumi di *Acacia eradenia* Bth., *R. Pienaarii* su foglie e piccioli di *Acacia caffra* Willd., *R. Halsei* su foglie e piccioli di *Acacia ataxacantha* DC., *R. modesta* su foglie di *Acacia stolonifera* Burch., *R. transvaalensis* su foglie di *Acacia detinens* Burch.

La struttura e le dimensioni dei capitoli e delle spore singole, il numero dei cistidii, la presenza e le dimensioni delle papille sembrano però sufficienti a distinguere la *Ravenelia* in esame dalle specie menzionate e giustificare l'istituzione di una nuova specie; tanto più che di questo genere — *Ravenelia* —, le cui specie presentano così netto endemismo e tanto alta specializzazione matricale, non si hanno finora segnalazioni su *Acacia laeta.*

Tranzschelia pruni-spinosae (Pers.) Diet. in Ann. Mycol., vol. XX, pag. 31 (1922); sub *Puccinia pruni-spinosae* Pers. in P. e H. Sydow, Monogr. Uredin., vol. I, pag. 484. Su foglie di Prunus persica Stokes coltivato, Oletta (Scioa), ca. 2400 m.s.m., 7/11/1938; e Bonga (Galla e Sidama), ca. 1700 m.s.m., 21/11/1938.

Masseeella Ciferrii n.sp.

Uredosoris hypophyllis, sparsis vel parum aggregatis, epidermide flavida diu tectis, demum erumpentibus, applanatis, μ 150—250 diam.; uredosporis sub-globosis vel angulato-globosis, echinulatis, chlorinis, μ 16—20 ⩗ 13—17, membrana tenui, usque μ 1 crassa, poris inconspicuis; teleutosoris raro hypophyllis, solitariis, praesertim epiphyllis, densiuscule hic inde aggregatis, sed non confluentibus, immersis, sacculiformibus, quandoque in altera foliorum pagina leniter nigrescente-bullata vix conspicuis, μ 140—160 ⩗ 150—170 diam., cirris sporarum filiformibus, sinuoso-contortis, divergentibus, ochraceo-brunneis, mm. 2—3,5 long. et μ 50—70 lat., praeditis; teleutosporis muco contentis, obovato-polygoniis, interdum clavulatis, sub-levibus, demum subtilissime punctulatis, dilute brunneo-aurantiacis, unicellularibus, μ 22—28 ⩗ 17—20, episporio ca. μ 3 crasso, poro apicali distincto.

Hab. in foliis Berchemiae yemensis Deflens (Rhamnaceae), Adamitullo (Galla et Sidama) ca. 1700 m.s.m., 21/1/1939.

Nel gennaio del 1939 in una missione compiuta sui laghi Zuai e Margherita, nelle immediate vicinanze della nuova Adamitullo, non molto lungi dal Suk-Suki, ma in ambiente aridissimo, la mia attenzione veniva attratta da alcuni arbusti a rametti brevemente spinosi, determinati poi dal Prof. A. FIORI come *Berchemia yemensis*, mostranti le piccole foglie ovato-ellittiche o sub-rotonde,

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1) Ringrazio vivamente Miss. E. M. DOIDGE per il gentile invio dei suoi importanti lavori sulle Uredinee del Sud Africa.

diffusamente clorotiche. Le foglioline, che erano ripiegate lungo il rachide fogliare, quasi duplicate, mostravano sulla pagina inferiore minutissime macchioline grigio-nerastre, qua e là cosparse di leggere convessità appena visibili.

Scostando le due metà ravvicinate del lembo fogliare, si notavano nello epifillo minuscoli cespitoli di processi filiformi fragilissimi, divergenti e contorti o ripetutamente ravvolti su loro stessi, di tonalità bronzeo-scura.

Detti filamenti che possono superare in lunghezza mm. 3 e in larghezza si aggirano tra μ 50 e 70, osservati a forte ingrandimento, si sono rivelati costituiti da una sostanza mucosa jalina, allo stato secco più densa e rifrangente negli strati periferici, che lentamente si gonfia e si disfà in acqua o in altri liquidi (lattofenolo bollente, glicerina, ecc.).

Detti filamenti fuoriescono da cavità otricolari o a sacco del tessuto fogliare, che si aprono largamente a maturità nella pagina superiore e occupano a volte tutto lo spessore del lembo, talora sporgendo appena inferiormente, di solito senza erompere. Queste cavità proligere sono tappezzate da un imenio di ife jaline intrecciate; dai lati poi si spingono nella cavità stessa ife scolorate, il contorno delle quali sfuma gradatamente all' apice, quasi in gelificazione, originanti probabilmente la massa mucosa che tiene unite le teleurospore. Dietel [Ber. Deut. Bot. Ges., vol. XIII, pagg. 332—335 (1895)], nel descrivere tali ife in *Masseeella capparidis* (Hobs.) Diet., notava che nel muco fuoriuscente dalle cavità suddette se ne osservano ancora i residui. Le teleutospore, che si differenziano qua e là alla superficie del fitto intreccio di ife sopra descritto, il quale si rinnova di continuo per un tempo assai lungo, sono immerse nella massa mucosa, non seriate nè catenulate, e sono unicellulari, di forma ovoide o sub-trigona, di tonalità castanea intensa, con poro germinativo apicale ben distinto e parete quasi liscia, minutamente punteggiata 1). Le teleutospore misurano μ 22—28 ⩔ 17—20. Gli uredosori minutissimi (μ 150—250), ipofilli, sparsi o gregari, lungamente coperti dall' epidermide, poi erompenti, a superficie piana, mellea, mostrano uredospore clorine, sub-globose, echinulate, a parete sottile, di μ 16—20 ⩔ 13—17. La forma presente non mostra evidenti differenze morfologiche dalla sopra detta *Masseeella capparidis*, descritta accuratamente da Massee [Grev., vol. XXI, pag. 35 (1892) sub *Cronartium capparidis* Hobs.], della quale però è ignoto lo stadio uredosporico e da *M. flueggeae* Syd. [Ann. mycol., vol. XVI, pag. 424 (1928)], di cui G. B. Cummins ha descritto picnidii ed ecidii [Ann. Mycol., vol. XXXV, pag. 103 (1937)].

La specie di Dietel però è stata ritrovata su una capparidacea e *M. flueggeae* su una euforbiacea; la *Cronartiacea* ora descritta è stata invece ritrovata su una ramnacea.

Si è ritenuta pertanto giustificata l'istituzione di una nuova specie e la si è dedicata al Prof. Raffaele Ciferri, direttore dell' Istituto di Botanica della Facoltà di Agraria dell' Università di Firenze.

Le segnalazioni delle due precedenti specie provengono quasi esclusivamente da regioni asiatiche [Belgaum (Hobson), Burma (in Butler, The fungi of India, pag. 60, 1931), Filippine (Sydow), India (in Tirumalachar M. J., Proc. Indian Acad. Sci., Sect. B., XVIII, pagg. 36—40, 1943; Id. New Phytologist, XLII, pagg. 45—48, 1943), ecc.]. Per quanto si sa, da altri continenti si avrebbero soltanto le segnalazioni di E. M. Doidge in Bothalia [vol II, parte I, pag. 193 (1927) e vol. III, parte IV, pag. 488 (1939)], relative a *Masseeella flueggeae*; delle quali segnalazioni la prima si riferisce alla forma uredosporica: *Uredo brideliae* (P. Henn. et Evans) Doidge (= *Aecidium brideliae* P. Henn. et Evans.).

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1) Anche nelle teleutospore di *M. flueggea.* Syd., Arthur [Philippine Journ. of Sc., vol. LXI, n. 4, pagg. 463—488 (1936)] ha messo in evidenza fini striature longitudinali dell'episporio.

La segnalazione di questa nuova *Masseeella* sembra dunque anche perciò interessante.

Hemileia Gardeniae — Thunbergiae (P. Henn.) Maublanc e Roger in Bull. Soc. mycol. de France, vol. L, pag. 202 (1934); sub *Uredo gardeniae — thunbergiae* P. Henn. in Bot. Ergebn. der Kunene-Sambesi Exped., pag. 160 (1902); sub *Hemileia vastatrix* Berk e Br. in P. e H. Sydow, Monogr. Uredin., vol III, pag. 209. Su foglie di Gardenia lutea Fresen., valle del Diddessa, 6/12/1938, e sponde del Nilo Azzurro presso Bar-Dar, 23/2/1939, ad altitudini di circa 1600—1850 m.s.m.

In macchie grigiastre di tonalità chiara si sono osservate abbondanti teleutospore di forma obconica schiacciata, brevemente pedicellate, papillate all' apice. Quivi si sono talora osservati inizii di germinazione.

Hemileia Hansfordii Syd. in Ann. Mycol., vol. XXXVII, pag. 198 (1939). Su foglie di Jasminum abyssinicum R. Br., presso Becca nel Commissariato dei Beni-Sciangul (Galla e Sidama), ca. 1650 m.s.m., 26/3/1939.

Nel materiale osservato le uredospore raggiungono dimensioni di *μ* 20—28 × 17—20, alquanto superiori a quelle data da Sydow (*μ* 17—26 × 15—17).

Hemileia vastatrix Berk. e Br. in Gard. Chron., pag. 1157 (1869); P. e H. Sydow, Monogr. Uredin., vol. III, pag. 209. Su foglie di Coffea arabica L., presso Bonga, 21/11/1938, Gimma, 10/12/1938, Aggaro, 4/12/1938, Gore, 9/12/1938 (Galla e Sidama), ad altitudini di circa 1600—1850 m.s.m.

Uromyces aloes (Cke) P. Magn. in Ber. Deutsch. Bot. Ges., vol. X, pag. 48 (1892); P. H. Sydow, Monogr. Uredin., vol. II, pag. 265; sub *Uredo aloes* Cooke in Grev., vol. XX, pag. 16 (1891); Sacc., Syll. fungor., vol. XI, pag. 227. Su foglie di Aloe sp., presso la palude dell' Auasa (Galla e Sidama), ca. 1500 m.s.m., 27/1/1939.

Di questa *Pucciniacea* si ha una segnalazione eritrea da parte di Canonaco [Boll. R. Giard. Colon. Palermo, vol. XIV, pag. 5 (1936)], su materiale con teleutospore a episporio liscio („*perfecte levi*"), come nella prima diagnosi di Cooke (loc. cit.). Nell' esemplare esaminato le teleutospore sono invece finemente verruculose, come nella descrizione del tipo in Monogr. Uredin., vol. II, pag. 265.

Uromyces cluytiae Kalchbr. e Cooke in Grev., vol. XI, pag. 20 (1883); P. e. H. Sydow, Monogr. Uredin., vol. II, pag. 153. Su foglie di Cluytia lanceolata Forsk., presso Gondar (Amara), ca. 1950 m.s.m., 28/2/1939.

Uromyces geranii (D.C.) Fries in Summa Veg. Scand., pag. 514 (1849); P. e H. Sydow, Monogr. Uredin., vol. II pag. 190. Su foglie di Geranium sp., Bonga (Galla e Sidama), ca. 1700 m.s.m. 23/11/1938.

Uromyces Hobsoni Vize in Grev., vol. IV, pag. 115 (1876); P. e H. Sydow, Monogr. Uredin., vol. II, pag 38. Su lembi e piccioli fogliari, rametti verdi, calici e peduncoli fiorali di Jasminum floribundum R. Br., nei pressi del fiume Angareb, vicino Gondar (Amara), ca. 1950 m.s.m., 27/2/1939.

Nell' esemplare esaminato, di indubbio riferimento specifico per „habitus" e per i caratteri della forma teleutosporica, gli ecidii hanno pseudoperidio lungamente cilindrico (ca. mm. 2 × 0,3), a margini lacerati leggermente revoluti. Il reperimento di questa forma in pieno sviluppo a stagione secca inoltrata è da porre in rapporto con le osservazioni di Barclay [Trans. Linn. Soc., 2 ser. Bot., vol. III, pagg. 141—151 (1891)] sulla germinazione delle ecidiospore e con i resultati ottenuti da Thirumalachar [Phytopat., vol. XXIX, pagg. 783—792 (1939)] sui rapporti tra ecidiosori e teleutosori.

Uromyces illotus Arth. e Holw. in Amer. Journ. of Bot., vol. V, pag. 441 (1918); Sacc., Syll. fungor., vol. XXIII, pag. 652. Su foglie di Mucuna rubro-aurantiaca De Wild., presso Bonga (Galla e Sidama), ca. 1600 m.s.m., 21/11/1938.

L'esemplare osservato concorda col tipo, segnalato su *Mucuna andreana* M. Micheli, per il comportamento e i caratteri morfologici delle uredo e teleutospore. Queste ultime però, pur avendo parete distintamente ispessita all' apice, sono di forma tondeggiante e misurano *μ* 20—24 ⩔ 16—21. La forma presente sembra pertanto ravvicinabile sotto questo riguardo ad *Uromyces mucunae* Rabh.

Puccinia abyssinica (P. Henn.) Syd. in Monogr. Uredin., vol. I, pag. 345; sub *Puccinia exhauriens* Thuem. var. *abyssinica* P. Henn. in Hedw., vol. XXXIV, pag. 329 (1985). Su foglie di Jasminum abyssinicum R. Br., Zeghiè sul lago Tana, ca. 1850 m.s.m., 24/2/1939.

Puccinia anomala Rostr. in Flora., vol LXI, pag. 92 (1878); sub *P. simplex* (Koern.) Erikss. in P. e. H. Sydow, Monogr. Uredin., vol. I, pag. 756. Su foglie di Hordeum sativum Pers. coltivato, tra Gimma e Santammà (Galla e Sidama), ca. 1750 m.s.m., 29/11/1938.

Puccinia coronata C.da in Icon. fung., I, pag. 6 (1837); P. e H. Sydow, Monogr. Uredin., vol. I, pag. 699. Su foglie di Avena sativa L. coltivata, Ambò (Scioa), ca. 2200 m.s.m., 7/11/1938.

Puccinia glumarum (Schm.) Erikss. e Henn. in Zeitschr. f. Pflanzenkr., vol. IV, pag. 197 (1894); P. e. H. Sydow, Monogr. Uredin., vol. I, pag. 706. Su foglie, culmi e spighe di Triticum vulgare Vill. coltivato, Ambò (Scioa), ca. 2300 m.s.m., 29/10/1938.

Puccinia graminis Pers. in Neues Mag. Bot., vol. I, pag. 119 (1794); P. e H. Sydow, Monogr. Uredin., vol. I, pag. 692. Su foglie e culmi di Triticum vulgare Vill. coltivato, Ambò (Scioa), ca. 2300 m.s.m., 29/10/1938.

Puccinia helianthi Schwein. in Schroet. Nat. Ges. Leipzig, vol. I, pag. 73 (1882); P. e H. Sydow, Monogr. Uredin., vol. I, pag. 92. Su foglie di Helianthus annuus L. coltivato, Malcò (Galla e Sidama), ca., 1800 m.s.m., 26/11/1938.

Puccinia rubigo-vera tritici (Erikss. e Henn.) Carl. in Arthur, Manual of the rusts U.S.A. and Canadà, Lafayette, pag. 181 (1934); sub *P. triticina* in P. e H. Sydow, Monogr. Uredin., vol. I, pag. 716. Su foglie e culmi di Triticum vulgare Vill. coltivato, Ambò (Scioa), ca. 2300 m.s.m., 29/10/1938.

Puccinia Schweinfurthii (P. Henn.) P. Magn. in Ber. Deutsch. Bot. Ges., vol. X, pag. 44 (1892); P. e H. Sydow, Monogr. Uredin., vol. I, pag. 446. Su foglie di Rhamnus prinoides l'Hérit., presso Gondar (Amara) ca. 1800 m.s.m., 28/2/1939.

Aecidium englerianum P. Henn. e Lindau in F. aethiop., I, pag. 110; P. e H. Sydow, Monogr. Uredin., vol. IV, pag. 256. Su giovani rami e foglie di Clematis Wightiana var. glaucescens Fres., presso Gondar, ca. 1950 m.s.m., 29/2/1939, e Clematis sp., Bunno-chelle, sulla pista di Gore (Galla e Sidama), ca. 1800 m.s.m., 4/12/1938.

Aexcidium rhamni Gmel. in J. F. Linnè, Syst. Nat., II, pag. 1472 (1791); P. e H. Sydow, Monogr. Uredin., vol. I, pag. 699. Su organi verdi di Rhamnus prinoides L'Hérit., Oletta (Scioa), ca. 2400 m.s.m., 12/11/1938.

L'ecidio si trova abbondantemente in aree lunghe anche 10 cm., ipertrofizzate, deformate, di bella colorazione giallo-rossastra su calici fiorali, giovani rametti, piccioli e lembi fogliari di „Ghescio" (denominazione amarica del *R. prinoides*). Gli ecidi possono trovarsi su ambedue le pagine fogliari e vi determinano galle o rigonfiamenti a vescica del parenchima nelle zone attaccate. Essi hanno forma dapprima cupulato-conica, poi a maturità colonnare con bordi laciniati, revoluti. Le ecidiospore globoso-poligonali, a parete echinulata incolora e contenuto plasmatico aranciato, misurano *μ* 14—18 ⩔ 16—24.

Poichè di questa forma ecidica vivente su *R. prinoides*, per quanto si sa non ancora segnalata, non è stato accertato alcun rapporto metagenetico con *Puccinia coronata* Cda [nel senso di Arthur, in Manual rusts U.S.A. and Canada, pag. 152 (1934)], si è creduto di doverla segnalare come *Aecidium rhamni*; denominazione comprendente le forme ecidiche a ciclo ancora ignoto, viventi su *Rhamnus* [P. Hariot, les Urédinées, pag. 290 (*Oecidium rhamni* Gml.)].

Aecidium Schweinfurthii P. Henn. in Bot. Ver. Brand., vol. XXX, pag. 299; P. e H. Sydow, Monogr. Uredin., vol. IV, pag. 108. Su rami di Acacia sp., presso il lago Zuai, ca. 1700 m.s.m., 1/2/1939.

Tilletia Baldratii Montem. in Boll. R. Giard. Col. Palermo, vol. XIII, pag. 44—46 (1934); E. Castellani e A. Ciccarone, malattie crittogamiche del teff in Ciferri e Baldrati, Cer. Afr. It., II, pagg. 83—86, R. Istituto Agr. Africa It., Firenze (1939). In ovarii di Eragrostis Teff (Zucc.) Trott., presso Mendi (Beni-Sciangul, Galla e Ṣidama), ca. 1650 m.s.m., 1/4/1939.

In alcuni dei nuovi esemplari osservati non si è notato un aumento ma piuttosto una riduzione delle dimensioni delle cariossidi.

Tilletia heterospora (P. Henn.) Zundel in Mycol., vol XIV, pag. 413 (1937); sub *Ustilago heterospora* P. Henn. in Pilze Oest Afr., pag. 48 (1895); Sacc., Syll. fungor., vol. XIV, pag. 413. In ovarii deformati di Setaria sp., concessione Borgnino (Collina isolata, Scioa), ca. 2200 m.s.m., 27/10/1938.

I fiori di *Setaria* colpiti da questa Ustilaginea sono globosi, talora un po' depressi, ipertrofici (3—5 mm di diametro) e a maturità si aprono superiormente per una o più rime, lasciando uscire la massa atra, polverulenta delle spore.

Questo "habitus" di facile riconoscimento del fungo è bene evidente solo nella diagnosi originale di P. Hennings.

Graphiola phoenicis (Moug.) Poit. in Ann. Scienc. Nat., vol. I, pag. 483 (1824); Sacc., Syll. fungor., vol. VII, pag. 522. Su foglie di Phoenix reclinata Jacq., presso Bonga (Galla e Sidama), ca. 1600 m.s.m., 21/11/1938.

Sorosporium Healdii Zundel in Mycol., vol. XXII, pag. 147 (1930); in infiorescenze di Hyparrhenia sp., pressi di Asosa (Beni-Sciangul, Galla e Sidama), ca. 1500 m.s.m., 19/3/1939.

I sori del parassita si curvano alquanto verso l'estremità assottigliata. La falsa membrana di colore bruno-giallastro ha cellule sterili a parete spessa, ialine, di forma approssimativamente cubica o allungata, misuranti *μ* 10—15 ⩔ 4—9. Deiscendo essa apicalmente, si liberano i glomeruli atri, frammisti a numerosi, sottilissimi processi nastriformi attraversanti longitudinalmente il soro; i quali glomeruli constano di numerose clamidospore, le esterne olivacee, sottilmente verruculose in immersione, le interne scolorate, poligonali, raramente granulose. Le spore oscillano, in dimensioni, entro limiti alquanto più ampi di quelli del tipo:

Diametro delle clamidospore in *μ*:	7	8	9	10	11	12	13	14
frequenza su 100 misurazioni	4	16	18	*28*	20	12	1	1

Andropogon cimbarius L., non A. Rich., su cui il fungo è stato segnalato da Zundel è sinonimo di *Hyparrhenia cymbaria* Stapf.

Sphacelotheca cruenta (Kuehn) Potter in Phytopat., vol. II, pagina 98 (1912); sun *Ustilago cruenta* Kuehn in Hamb. Gart. u. Blumenztg., vol. XXVIII, pag. 177 (1872); Sacc., Syll. fungor., vol. VII, pag. 455. In infiorescenze di Sorghum durra (Hakl.) Stapf.[1] coltivato, Bonga (Galla e Sidama) ca. 1800 m.s.m., 21/11/1938.

Sphacelotheca elyonuri n.sp.

Sori ovarium colentibus et id destruentibus, 2—3 mm, longis 0,5—1 mm crassis, vel totam inflorescentiam occupantibus et tunc 1—5 mm longis, 2—4 mm crassis, pseudomembrana flavobrunnea e matricis cellulis fungique prosoplectenchimate intermixtis constituta; columella matricali centrali crassa, bene evoluta, extus nigra et mycelio induta; massa sporarum subatra, compactiuscula, granulosa vel pulverulenta; sporis saepe initio laxe breviter catenulatis et tunc, per aggregationem laxam hypharum parallelarum sporigenarum, glomerulos parallelepipedos simulantibus, postea liberis, subsphaericis, castaneis, 5—9 μ diam. (pro more 6—8 μ diam.), episporio minutissime punctulato, crassiusculo, circiter μ 1 metiente, praeditis; cellulis hyalinis, forma et magnitudine sporis non dissimilibus, raris.

Hab. in ovariis et in inflorescentiis Elyonuri argentei Nees var. caespitosi Hack. (Graminaceae), Asosa et Afodu (Galla et Sidama), ca. 1600 m.s.m., 19/3/1939 et 28/3/1939.

Questa Ustilaginacea è stata raccolta ad Afodu ed in due altre località più vicine ad Asosa. Le alterazioni dei singoli ovari si notarono senza grave difficoltà nelle formazioni di graminacee dominate da questo *Elyonurus*, che impartiva loro una tonalità argentea uniforme. Gli ovari sono completamente occupati e distrutti dai sori, che traspaiono a maturità attraverso le glume pellucide. Essi si mostrano atri per l'irregolare deiscenza della pseudomembrana, di cui persistono solo brandelli irregolari alla base. Negli esemplari esaminati, vecchi di circa 10 anni, la columella, inferiormente dilatata a cono ed occupante completamente la base dello ovario deformato, si prolunga poi di circa 2 mm (non superando complessivamente in genere i 3 mm di lunghezza) nell'estremità distale, che si assottiglia più gradualmente e che negli esemplari esaminati termina mozza. Macroscopicamente i sori si presentano talora compatti, non polverulenti.

Quando l'intera infiorescenza è investita, il soro si mostra lungamente cilindrico (fino ad oltre 5 cm) e di mm 1—2 di diametro. La pseudomembrana, costituita da un prosoplectenchima non ben definito frammisto a residui dei tessuti matricali, è colorata e tende alla terra d'ombra. La columella, che occupa gran parte del lume del soro, è crassa e longitudinalmente plicata; allontanati i conidi, si presenta di colore ombrino od alutaceo sporco. I clamidoconidi sono stati talora osservati, specie quando l'intera infiorescenza è attaccata, aggregati in masse parallelepipede lasse di ife sporigene strettamente avvicinate e contemporaneamente differenzianti i conidi in file parallele. Questi pseudoglomeruli però si disfanno a maturità e lasciano liberi i clamidoconidi, singoli e subsferici, non poligonali. Su 60 misurazioni, i clamidoconidi misurarono in media µ 6,5—7,5 (max µ 8,8; min. µ 5); essi sono di color castaneo intenso, di contenuto uniforme, trasparente, e presentano parete di circa µ 1, minutamente punteggiata.

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[1] Dei diversi sorghi coltivati nell'Africa tropicale e sub-tropicale [*Sorghum caffrorum* Beauv., *S. caudatum* Stapf., *S. cernuum* Host., *S. Roxburghii* Stapf., *S. durra* (Hakl.) Stapf.] si crede che il più delle volte quelli coltivati nell.interno dell' Africa Orientle possano essere riferiti a *S. durra*; benchè certo altre specie, come sembra indicare anche la varietà delle denominazioni locali in uso, in particolare *S. candatum*, non sieno assenti dai territori dell'Africa Orientale.

Come è noto per gli *Ustilaginales* in particolare il giudizio sistematico può essere fondato su due criteri opposti: il primo che tende a riconoscere un minimo numero di entità specifiche, l'altro che vorrebbe attribuire dignità di specie a caratteri anche biologici in genere ritenuti di importanza minore. Della prima tendenza, nel campo dei carboni, sembra sostenitore Zundel, delle seconda sono, ad esempio, Liro e Ciferri.

Zundel però nelle sue chiavi dicotomiche integra talora il criterio della specializzazione del parassitismo o ad esso sostituisce parzialmente quello della elettività. Nel nostro caso, così, accettando *Sphacelotheca andropogonis* (Opiz) Bubak nel senso di Zundel, estendone cioè il parassitismo ai generi *Andropogon*, *Cymbopogon*, *Ischaemum* e *Pennisetum*, potremmo riferire ad essa quella parte del materiale in cui tutta l'infiorescenza è occupata dal parassita. Si verrebbe però a dover escludere econsiderare nuova entità specifica il materiale ovaricolo. Come è noto, questo criterio non sembra sempre condiviso, per la diversa localizzazione di uno stesso carbone su diverse specie e, fra gli individui parassitati, nell' ambito di una stessa specie. Per l'*Elyonurus* in esame, raccolto tutto in località limitrofe ed ecologicamente vicinissime, si è notato che i sori ovaricoli — che apparirebbero nettamente distinti dagli altri, se considerati singolarmente — possono essere presenti sullo stesso individuo, sul quale altre infiorescenze sono completamente distrutte e talora si differenziano nella metà di un'infiorescenza che, per il resto, è parassitata in tutti i suoi organi. La diversa apparenza potrebbe dunque essere messa in rapporto con modalità di sviluppo del parassita e soprattutto dell' ospite, in conseguenza di fatti ambientali. Si è notata ad esempio abbondanza di sori parassitanti l'intera infiorescenza, laddove la fioritura osservata seguiva sull 'infiorescenza stessa si osservavano le tracce dello incendio del pascolo comunemente eseguito dagli indigeni durante la stagione secca. L'attento esame morfologico e le misure eseguite sui due tipi di sori hanno confermato la presunzione che essi siano da considerare come manifestazioni distinte di uno stesso carbone; carbone che, per l'aspetto macroscopico, la natura della pseudomembrana, la presenza di columella e di clamidoconidi liberi, è stato attribuito al genere *Sphacelotheca* (di cui ricorda da vicino, specie nell' aspetto esterno, *S. andropogonis*) col nome di *S. elyonuri.*

Sphacelotheca sorghi (Link.) Clinton in Journ. Myc., vol. VIII, pag. 140 (1902); sub *Ustilago sorghi* Pass. in N. G. Bot. It., vol. LX, pag. 235 (1877); Sacc., Syll. fungor., vol. VII, pag. 456. In infiorescenze di Sorghum durra (Hakl.) Stapf. coltivato, Becca (Beni-Sciangul, Galla e Sidama), ca. 1600 m.s.m., 27/3/1939.

Tolyposporium cenchri Bref. in Unters., vol. XII, pag. 156; Sacc., Syll. fungor., vol. XIV, pagg. 425—426 (1899). In infiorescenze di Cenchrus ciliaris L., presso il Lago Zuai (Galla e Sidama), ca. 1700 m.s.m., 1/2/1939.

Il riferimento di questa specie è complicato dalla molteplicità dei carboni già segnalati sul genere *Cenchrus* e dalle diverse opinioni degli Autori.

Sembra tuttavia di poter escludere *Ustilago Cenchri* Lagh., trovata in Equatoria, per il carattere "*spicam inclusam destruentibus*". Più incerta è la determinazione dei caratteri specifici di *Sorosporium Cenchri* P. Henn. [in Hedw., vol. XXXV, pag. 221 (1896)]. I caratteri diagnostici di questa specie la avvicinano strettamente a *Tolyposporium Cenchri.* Il solo carattere differenziale da me notato, "*intus punctulatis*", non sembra di importanza massima.

Ero anzi dell' opinione di avvicinare i miei esemplari a *Sorosporium cenchri*; ma ne sono stato trattenuto, per essere esso da Clinton [N. Am. Flora, vol. VII, Parte I, pag. 38 (1906)] messo in sinonimia con *Sorosporium syntherismae* (Peck) Farl., trovato su varie specie di *Cenchrus* e *Panicum* e nettamente diverso dal fungo ora in esame (forma dei sori, modalità di apertura di essi, presenza di columella). Le misure delle clamidospore non sono di molto giovamento al proposito [vedasi anche: Ciferri R., Quinta contribuzione allo studio degli Ustilaginales, Ann. Mycol., vol. XXIX, pagg. 1—74 (1931)].

Tolyposporium cenchri è descritto nella diagnosi originale con „*sporis brunneolis, non verrucosis*". Gli esemplari esaminati sono talora punteggiati o verrucosi su tutta o su parte della superficie esterna dell' episporio.

Su 100 clamidoconidi, le misure del diametro dettero le frequenze seguenti:

Diametro dei clamidoconidi in μ:	< 9,5	9,5—10,4	10,5—11,4	11,5—12,4	
Frequenza percentuale:	6	16	*66*	12	= 100

Ustilago avenae (Pers.) Jensen, Charb. des céréals, pag. 4 (1889); Sacc., Syll. fungor., vol. IX, pag. 283. In infiorescenze di Avena sativa L., coltivata, Malcò, presso Gimma (Galla e Sidama), ca. 1700 m.s.m., 26/11/1938.

Ustilago cynodontis (Pass.) P. Henn. in Engl. Bot. Jahrbuch., vol. XIV, pag. 369 (1891); Sacc., Syll. fungor, vol. XIV, pag. 496. Su infiorescenze di Cynodon dactylon Pers., Oletta (Scioa), ca. 2400 m.s.m., 7/11/1938.

Ustilago hordei (Pers.) Lagerheim in Mitt. d. Badischen Bot. Ver., pag. 70 (1889); sub *U. hordei* (Pers.) Kell. e Sw. in II Ann. Rept. Kansas Agric. Exp. Stat., pag. 268 (1890); Sacc., Syll. fungor., vol. IX, pag. 283. In infiorescenze di Hordeum sativum Pers. coltivato, Debra-Berhan (Scioa), ca. 2600 m.s.m., 28/10/1938.

Ustilago linearis (Dozy e Molkenboer) Ciferri in Fl. It. crypt., Pars I, Ustil., pag. 339 (1938). Su culmi di Hyparrhenia cymbaria Stapf (= Andropogon cymbarius L., non A. Rich.), presso Soccurrù, sulla strada di Addis Abeba a Gimma (Galla e Sidama), ca. 1900 m.s.m., 14/11/1938.

Ustilago tritici (Pers.) Jensen in Ann. Rept. Kansas Agr. Exp. Stat., II, pag. 262 (1890); Sacc., Syll. fungor., vol. IX, pag. 283. In infiorescenze di Triticum vulgare Vill. coltivato, Biscioftu (Scioa), ca. 1900 m.s.m., 17/10/1938.

Ustilago vittata Berk. in Hook. Journ., vol. VI, pag. 206 (1854); Sacc., Syll. fungor., vol. VII, pag. 459. In ovarii di Oplismenus compositus P. Beauv., Malcò (Galla e Sidama), ca. 1700 m.s.m., 18/11/1938.

Diametro delle clamidospore in μ:	15	16	17	18	19	20
frequenza su 100 misurazioni:	1	2	21	48	18	10

Le dimensioni delle spore coincidono con quelle date da Berkeley (loc. cit.) e riportate nella Sylloge, mentre sono alquanto superiori a quelle riportate da Fischer De Waldheim (Aperçu sistém. des Ustilag., pag. 18) sulla stessa specie (μ 14—18).

L'episporio invece viene descritto da Berkeley come liscio, ma da Fischer „à granulations très prononcées"; e così si presenta nello esemplare etiopico.

Non si sono mai osservate spore pedicellate.

Phyllosticta divergens Sacc. in Fungi abyss. n. 35 [Malpighia, vol. V, pag. 274 (1891)]; Sacc., Syll. fungor., vol. X, pag. 138. Su foglie di Albizzia schimperiana Oliv., Malcò, presso Gimma (Galla e Sidama), ca. 1750 m.s.m., 18/11/1938.

Phyllosticta gardeniae F. Tassi in Bull. del Lab. Orto Bot. Siena, vol. III, pag. 54 (1900); Sacc., Syll. fungor., vol. XVI, pag. 840. Su foglie di Gardenia lutea Fresen., pressi del Nilo Azzurro (alle bocche del lago Tana), ca. 1750 m.s.m., 26/2/1939; socia di Sphaerella gardeniae Cooke.

La presente *Phomacea* è con tutta probabilità identica a *Phyllosticta gardeniae* Cooke (*nomen nudum*) in Journ. of Bot., pag. 108 (1883) che questo autore ritenne stato spermogonico della *Sphaerella* sopra menzionata.

Phyllosticta gymnosporiae n.sp.

Maculis amphigenis, primo luteo-ochraceis, dein magnis (usque 3 cm latis) angulosis, sub-orbicularibus, albido-cinereis, leniter zonatis, aridis, margine brunneo-rufescente, vix prominulo cinctis, interdum fissis; pycnidiis amphigenis, plerumque epiphyllis, epidermide velatis, sparsis, globoso-depressis, μ 90—120 diam., poro μ 18—25 diam. pertusis, contextu membranaceo, fuligineo, obscure pseudo-parenchymatico; sporulis cylindraceis, utrinque rotundatis, rectis, continuis, μ 8—10 ⩔ 3—4.

Hab. in foliis Gymnosporiae sp. (Celastraceae), prope Bonga (Galla et Sidama), ca. 1800 m.s.m., 23/11/1938.

Sulle foglie di *Gymnosporia* sp. sono state più volte osservate in diverse località umide dell' occidente dell' Impero, macchie dapprima piccole, ferruginee, indeterminate, poi assai più grandi, bianco-cenerine al centro e marginate di rosso scuro; detto margine sfuma all' esterno in un alone rossastro più o meno esteso. Le macchie sono densamente punteggiate da minuscoli picnidii sottoepidermici appena erompenti quando sono maturi per la sommità ostiolata (l'ostiolo misura μ 18—25) e di forma generalmente globoso-depressa, misuranti μ 90—120 di diam. I picnidi contengono sporule cilindracee, leggermente verdognole, continue, di μ 8—10 ⩔ 3—4. Questo fungillo è molto dannoso alle piante attaccate.

Phyllosticta heveae Zimm. in Bull. Inst. Bot. Buitenz., pag. 21 (1901); Sacc., Syll. fungor., vol. XVIII, pag. 223. Su foglie di Hevea brasiliensis Muell., Saddu (Galla e Sidama), ca. 1300 m.s.m., 15/12/1938.

Phyllosticta manihot Speg. in An. Mus. Buenos Aires, vol. XX, pag. 335 (1910); Sacc., Syll. fungor., vol. XXII, pag. 832. Su foglie di Manihot Glaziovii Muell. coltivato, Saddu nella valle del fiume Baro (Galla e Sidama), ca. 1300 m.s.m., 15/12/1938.

Phyllosticta Stevensii Young in Mycol., vol. VII, pag. 147 (1915); Sacc., Syll. fungor., vol. XXV, pag. 76. Su foglie di Triumphetta sp., Urumu (Galla e Sidama), ca. 1800 m.s.m., 10/12/1938.

Phyllosticta visci (Sacc.) Allesch. in Fungi imperf., Abt. VI, pag. 96 (1901); sub *Phoma visci* Sacc. in Michelia, vol. I, pag. 125; Sacc., Syll. fungor., vol. III, pag. 113. Su foglie e giovanissimi assi di Viscum sp., Gondar (Amara), ca. 1950 m.s.m., 27/1/1939.

Phoma balanitis n.sp.

Maculis apicalibus, depressis, arescentibus, cinereo-luteis, infra ochraceo-marginatis; pycnidiis sparsis vel gregariis, immersis, atris, interdum in spatio hypostomatico sitis, μ 100—160 diam., irregulariter globoso-conoideis, carbonaceo-coriacellis, ostiolo irregulari, epidermide perforante, praeditis, μ 20—30 diam., contextu opaco, indistincto; sporulis subglobosis vel bacillaribus, continuis, hyalinis, μ 2—6 ⩔ 1,5—2,5.

Hab. in spinis adhuc vivis Balanitis aegyptiacae Del. (Zygophyllaceae), Bolè, prope lacum Zuai (Galla et Sidama), ca. 1700 m.s.m., 21/1/39.

Questo fungillo, che si osserva sulle spine di *Balanitis aegyptiaca* produce depressi essiccamenti apicali, circondati da un sottil margine di tonalità rugginosa, sfumante inferiormente in un alone gialliccio. Sulle macchie sono visibili minutissimi punti neri assai fitti: i picnidii del fungo. Detti picnidii, di forma irregolare, carbonacei, ostiolati, immersi, di μ 100—160, si formano spesse volte nella camera sottostomatica e si aprono all' esterno mediante un ostiolo papillato, affiorante alla superficie dell' epidermide, ma non prominente. Le sporule ialine, di varia forma, da globose a bacillari, sono minutissime, misurando circa μ 2—6 × 2. Il micete a quanto sembra accelera i processi normali di decadimento delle spine.

Phoma theicola Petch in The Dis. of the Tea bush, pagg. 42 e 209 (1923). Su foglie di Thea sinensis L. coltivata, Bonga (Galla e Sidama), ca. 1750 m.s.m., 21/11/1938.

Aposphaeria eragrostidis Ciccar. e E. Castell., Malattie crittog. del Teff. in Ciferri e Baldrati, Cer. Afr. It., II, pagg. 99—101, R. Ist. agron. Afr. It., Firenze (1939). Su glume e cariossidi secche di Eragrostis Teff (Zucc.) Trott., Mendi (Beni-Sciangul, Galla e Sidama), ca. 1650 m.s.m., 1/2/1939.

Darluca filum (Biv.) Cast. in Catal. Pl. Marseill., Supplem., pag. 53; Sacc., Syll. fungor., vol. III, pag. 410. In uredosori e teleutosori diversi, raccolti in più località dell' Etiopia.

Nei teleutosori di *Puccinia abyssinica* (P. Henn.) Syd. su *Jasminum abyssinicum* R. Br. si è osservata una *Darluca* avente picnidii densamente stipati, di aspetto cespitoso, quasi stromatico. Questa forma sarebbe forse vicina a *Darluca genistalis* (Fr.) Sacc.

Septoria balanitis n.sp.

Maculis amphigenis, sparsis, rotundatis, raro confluentibus, usque 3 mm latis, primo flavidis dein arescendo albidis, margine prominente rubro-brunneo cinctis; pycnidiis amphigenis, in centro macularum solitariis vel gregariis, punctiformibus, epidermide velatis, demum erumpentibus, nigris, membranaceis, globoso-depressis, μ 140—200 diam., maturitate late pertusis; sporulis filiformibus, nubilosis, flexuosis, utrinque attenuatis, obsolete guttulatis, continuis vel medio I-septatis, μ 12—35 × 2—3.

Hab. in foliis Balanitis aegyptiacae Del. (Zygophyllaceae), Bolè, prope Moggio (Galla et Sidama), 1700 m.s.m., 1/2/1939.

Le macchie biancastre, circoscritte da un sottile orlo elevato rosso-bruno, che il parassita produce sulle foglie, sono talora abbondantissime. Se dette macchie non sono sterili, come spesso avviene, l'epidermide dopo qualche tempo si solleva e si screpola al centro di esse, lasciando così erompere un solo, due o tre picnidi globosi, elissoidali, talora per mutua pressione deformantisi, membranacei, di μ 140—200. Detti picnidi a maturità si aprono per una larga fessura, emettendo talora in masserelle grumose sporule grigio-azzurrine, filiformi, vermicolari, granulose, indistintamente unisettate al mezzo ed ivi talvolta alquanto ristrette. Data la specializzazione del parassitismo in questo genere di deuteromiceti più volte messa in luce dagli studiosi [W. S. Beach in Amer. Journ. of Bot., vol. VI, pagg. 1—33 (1919); G. F. Weber in Phytopath., vol. XII, pagg. 449—470 (1922); id., vol. XII, pagg. 537—585 (1922) e vol. XIII. pagg. 1—23 (1923); R. Sprague in Mycol., vol. XXX, pagg. 672—678 (1938)], nè avendosi segnalazioni di *Septoriae* sul gener. *Balanites* Del., si è creduto di poter descrivere la forma osservata come nuova.

Septoria lycopersici Speg. in F. Argent., pag. IV, n. 289; Sacc., Syll. fungor., vol. III, pag. 535. Su foglie di Lycopersicum esculentum Mill. coltivato, Maccanise, presso Addis Abeba, ca. 2300 m.s.m., 21/10/1938.

Septoria proteae n.sp.

Maculis amphigenis, subcircularibus, mm 3—4 diam., saepe confluentibus, primo laete castaneis, dein cinerascentibus, elevato-marginatis; pycnidiis amphigenis, plerumque in maculis solitariis, quandoque 2—3 sparsis, immersis, demum ostiolo erumpentibus, atris, globosis, μ 120—140 diam., contextu membranaceo, distincte pseudo-celluloso, obscure olivaceo; sporulis filiformibus, rectis vel curvulis, dilute brunneolis, altero apice acutato, 1—3 septatis, μ 40—50 ⩔ 3—4.

Hab. in foliis vivis Proteae abyssinicae W. (Proteaceae) in locis siccis vallis amnis Diddessa (Galla et Sidama), ca. 1700 m.s.m., 3/12/1938.

Questa septoriosi, largamente diffusa sulle foglie della sola specie di *Protea* presente in Etiopia, è facilmente riconoscibile dai caratteri macroscopici. Il parassita difatti produce macchie rotondeggianti, regolari, di tonalità sfumante dal castaneo chiaro al testaceo e al cinereo, a margine rilevato molto evidente, al centro delle quali si trova di solito un sol picnidio immerso, appena visibile, quando è maturo, ad occhio nudo, per l'ostiolo erompente. I picnidi globosi, membranacei, di μ 120—140 di diam., emettono a maturità picnospore quasi sub-clavulate, a estremità basale arrotondata, apice lungamente acuminato, e di colore scuro, olivaceo-verdognolo. Sul genere *Protea* L. non si hanno segnalazioni di *Septoria*.

Septoria rosae Desm. in Exs. edit. I, ser. I, n. 535; Sacc., Syll. fungor,. vol. III, pag. 485. Su foglie di Rosa abyssinica R. Br., presso il fiume Angareb, Gondar (Amara), ca. 1950 m.s.m., 1/3/1939.

Septoria syzigii n.sp.

Maculis amphigenis, epiphyllis atro-sanguineis, hypophyllis luteo-ochraceis, mm 2—5 latis, polygoniis, nervis limitatis, quandoque confluentibus, margine elevato-purpureo; pycnidiis, μ 110—150 diam., praesertim epiphyllis, sparsis, plerumque sub-globosis, interdum vero e latere depressis, sub-epidermicis, maturitate poro usque μ 60 lato vix prominulis, sporulas globulo albido expellentibus, contextu crasso, membranaceo, indistincte (pseudo-) celluloso; sporulis filiformibus, curvulis, utrinque obtusis, continuis, chlorinis μ 30—40 ⩔ 3—4.

Hab. in foliis Syzigii guineensis DC. (Myrtaceae), prope alveum fluminis Angareb, Gondar (Amara), 1950 m.s.m., 20/2/1939.

Il parassita produce sulle foglie di questo *Syzigium* macchie poligonali internervali anfigene, visibilissime per la colorazione fondamentalmente rossastra, assai diversa da quella dei tessuti circostanti. I picnidii, sparsi in esse senza regolarità, hanno forma sub-sferica non ben definita, parete indistintamente pseudo-cellulare, e a maturità, aprendosi, fessurano largamente l'epidermide ed emettono le spore in massa gelatinosa e biancastra. Questo globulo rimane per lungo tempo trattenuto tra i lembi dell' epidermide rotta, sollevati a tronco di cono sul picnidio erompente. Le sporule vermicolari, sub-cilindriche, alle estremità ottuse, sono continue, a plasma omogeneo, di tonalità verde-azzurrina.

Phleospora olettae n.sp.*

Maculis amphigenis, orbicularibus, mm 3—8 latis, griseo-alutaceis, elevatis, induratis, interdum parce zonatis, saepe confluentibus, margine prominente cinctis; pseudo-pycnidiis plerumque epiphyllis, gregariis, sub-epidermicis, μ 60—130 diam., initio hemisphaericis vel lenticularibus, demum late erumpentibus, quandoque omnino applanatis, sporularum glomerulum laete carneum eructantibus, contextu pseudoparenchymatico indistincto, brunneolo; sporulis cylindraceis, sed plerumque utrinque longe attenuatis, rectis vel arcuatis, flexuosis, hyalinis, guttulatis, 2—5 septatis, non constrictis, raro in septis secendentibus, µ 22—32 ⩗ 2,5—3,5.

Hab. in foliis vivis Loranthi sp. (Loranthaceae), Oletta (Scioa), ca. 2400 m.s.m., 9/11/1938.

Il fungillo, assai comune in tutto l'ovest etiopico, produce macchie giallo-grigiastre, talora rossastre, sollevate sul lembo fogliare, che può esserne in gran parte coperto. Al centro di dette macchie, il parassita forma fruttificazioni sub-epidermiche, lenticolari, poi largamente erompenti. Le fruttificazioni mature sono talora più immerse, infundiboliformi o a coppa, talora invece del tutto appianate, con sporule disposte quasi verticalmente e parallele tra loro su uno strato proligero uniforme. Dette sporule, a profilo irregolare, cilindracee o più spesso fusoidee, talvolta si spezzano in due-tre parti in corrispondenza dei setti.

Il micete, ravvicinabile qualche volta a *Septoria* Fr., è forse più spesso a maturità vicino a *Septogloeum* Sacc. Esso è stato però riferito a *Phleospora* Wallr. per il diverso modo di presentarsi di fruttificazioni meno patenti e piuttosto immerse nei tessuti matricali.

In Argentina è stata segnalata da Spegazzini [in Anal. Mus. Nacion. Buenos Aires, vol. XX, pag. 384 (1919)] su *Loranthus ligustrinus* Wall. una *Septoria loranthicola,* riportata nella Sylloge (vol XXII, pag. 1112) con un punto d'interrogazione (*Septoria? loranthicola* Speg.). Benchè non sia stato possibile esaminare ,,exsiccata" di questa *Septoria,* sembra tuttavia di dover notare che per comportamento, caratteri morfologici, dimensioni, la *Septoria loranthicola* e la presente *Phleospora* sembrano coincidere assai bene, per quanto può desumersi dalla diagnosi della prima.

Colletotrichum gloeosporioides Penz. in F. agrum., II, pag. 6 (1882); Sacc., Syll. fungor., vol. III, pag. 735. Su foglie di Citrus bigaradia Duh., Gumaro presso Gore, ca. 1800 m.s.m., 16/12/1938.

Scolecosporium syzigii n.sp.

Acervulis amphigenis, plerumque hypophyllis, subcutaneo-erumpentibus, epidermide fissa cinctis, pulvinatis, applanatis, atris, subrotundatis vel polygoniis, µ 300—600 diam., saepe late confluentibus; conidiis 2—6 locularibus, castaneo-fuscis, obtuse verruculosis, infra rotundatis, sursum longe attenuatis vel prorsus rostratis, ibique sub-hyalinis, µ 40—60 ⩗ 6—8; conidiophoris dense stipatis, cylindraceis, parum septatis, concoloribus, µ 20—30 ⩗ 6—8.

Hab. in foliis Syzigii guineensis DC. (Myrtaceae), prope fluminem Omo-Bottego, ca. 1700 m.s.m., 25/1/1939; socia Micronectriae syzigii.

La presente forma, diversa per caratteri morfologici dalle altre specie di questo genere, si riscontra anfigena, ma sopratutto sulla pagina inferiore fogliare, di *Syzigium guineense,* e in particolare sui margini delle macchie di secco nelle quali si riscontra la *Micronectria* precedentemente descritta; macchie che lo *Scolecosporium* sembra estendere. Gli acervuli spesso confluenti del micete attirano l'attenzione dell' osservatore per l'intensa tonalità scura e le dimensioni notevoli (anche di varii millimetri, se gli acervuli confluiscono). Lo stroma, di colorazione sfumante dal testaceo alla terra d'ombra, erompe largamente a maturità e si solleva sull' epidermide, i cui lembi lacerati lo cingono. Gli acervuli maturi sono pulvinati e mostrano superficie piana, vellutata. I conidiofori, che si sollevano dallo strato proligero sono strettamente raddicinati, diritti, cilindrici, unisettati alla base, talora due-tresettati, arrotondati all' apice. I conidii, 2—6 loculari, di solito bisettati, intensamente castanei, a base arrotondata, si sollevano verticalmente dai conidiofori sui quali si inseriscono per un ilo ben evidente. Essi hanno parete ornata di verrucule ottuse, sparse, particolarmente frequenti nella porzione mediano-basale, e, cilindracei per la metà o i due terzi inferiori, si attenuano gradualmente e lungamente all' apice, arcuandosi spesso in maniera evidente in questa porzione superiore. Questo *Scolecosporium* è in genere socio di *Micronectria syzigii* (vedi ivi).

Pestalozzia caudata Sydow in Bull. l'Herb. Boiss., II ser., vol. I, pag. 84 (1901); Sacc., Syll. fungor., vol. XVI, pag. 1017. Su brattee fiorali di Cyperus papirus L., sponde del Nilo Azzurro nei pressi del lago Tana, ca. 1850 m.s.m., 25/2/1939.

Secondo la descrizione di Sydow, la lunghezza delle spore raggiunge μ 36—45. Le misure date da Guba [Mycol., vol. XXIV, pag. 363 (1932)] invece sono inferiori (μ 28—36); e quelle date da Grove [Brit. stem and Leaf-fungi, vol. II, pag. 349 (1937)] lo sono ancora di più (μ 28). Nell' esemplare esaminato dunque le spore sarebbero eccezionalmente sviluppate, oscillando in dimensioni da μ 37 a 55 (moda su 100 misurazioni = μ 45). La perfetta corrispondenza dei diversi caratteri morfologici dell' esemplare esaminato con quelli assai singolari del tipo sembra però non lasciar dubbi sul riferimento specifico.

Pestalozzia theae K. Sawada in Mycol., vol. IX, pag. 171 (1917); Sacc., Syll. fungor., vol. XXV, pag. 607. Su foglie di Thea sinensis L., Bonga (Galla e Sidama), ca. 1750 m.s.m., 22/11/1938.

Pestalozzia versicolor Speg. in Michelia, vol. I, pag. 479 (1879); Sacc., Syll., fungor., vol. III, pag. 790. Su foglie di Protea abyssinica W., valle del fiume Diddessa (Galla e Sidama), ca. 1600 m.s.m., 3/12/1938.

Questa *Pestalozzia* si è formata su vecchie foglie, morte e scolorate di *Protea abyssinica*, parassitizzate da *Septoria proteae* n.sp. (vedi ivi). Le fruttificazioni pseudo-picnidiche, anfigene, subepidermico-erompenti, di μ 120—140, sono limitate da una parete stromatica, che le ricopre in gran parte. A maturità l'epidermide lacerata si solleva a tronco di cono; e le spore sono emesse in globulo atro, convesso, quasi emisferico. Queste (μ 20—28 ⩔ 6—7) sono quinqueloculari ed hanno cellula inferiore (μ 4) regolarmente conoidea e cellula superiore obconica o più raramente tronco-conica, coronata di solito da tre ciglia filiformi, non rigonfiate all' apice e divergenti (circa μ 18—22). I loculi mediani, misuranti μ 15—18, sono di diversa tonalità: l'inferiore di tenue colorazione fumosa, i due superiori olivacei, il mediano assai intensamente colorato. Nonostante non si abbiano senalazioni di questa *Pestalozzia* su *Protea* o su generi vicini, si è creduto di poter riferire ad essa in base a questi caratteri la forma osservata. *P. versicolor* Speg., riscontrata nel tipo su foglie di *Nerium Oleander* L. cadute e in decomposizione, è stata ritrovata poi dallo stesso [f. *vagans* Speg., in Rev. Mus. de La Plata, vol. XIV, pag. 42 (1908)] su foglie in decadimento di molti alberi e arbusti ed è stata inoculata da Klebahn [Myc. Cblatt., vol. III, pagg. 109—115 (1913); id., vol. IV, pagg. 109—115 (1914)] con successo su diverse matrici, ma solo sotto campana e non in serre aperte: per cui anche questo autore la ritiene saprofita polifago [1]).

Ovulariopsis erysiphoides Pat. e Hariot in Journ. de Bot., vol. XIV, pag. 245 (1900); Sacc., Syll. fungor., vol. XVI, pag. 1936. Su foglie di Erythrina sp., Soddu (Galla e Sidama), ca. 2000 m.s.m., 27/1/1939; e su foglie di

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[1]) Guba però [Phytopath., vol. XIX, pagg. 22 (1929)] non ritiene esatto il riferimento specifico del materiale usato da Klebahn.

Dodonaea viscosa Jacq., presso Gondar (Amara), ca. 1950 m.s.m., 28/2/1939.

La forma conidica riscontrata su *Erythrina* dovrebbe riferirsi a *Phyllactinia suffulta* Sacc. f. *erythrinae* Jaczewsky [Monografia delle Erisifacee (in russo), pag. 436, Leningrado (1927)]. Della seconda matrice invece non si avevano precedenti segnalazioni.

Oidium erysiphoides Fr. f. *cordiae* Sacc. in Ann. Mycol., vol. VIII, pag. 339 (1910); Sacc., Syll. fungor., vol. XXII, pag. 1250. Su foglie di Cordia abyssinica R. Br., in luoghi e in stagioni diversi (Bonga, 21/11/1938; Gore, 12/12/1938; Soddu, 25/1/1939; Zeghiè, 24/2/1939; Becca, 26/3/1939; Shambo, 10/5/1939), ad altezze comprese fra circa 1600 e 2200 m.s.m.

Oidium leucoconium Desm. in Ann. Sc. Nat., ser. IV, vol. XIII, pag. 102 (1859); Sacc., Syll. fungor., vol. IV, pag. 41. Su foglie di Prunus persica Stokes coltivato, Addis Abeba, ca. 2500 m.s.m., 6/1/1939.

Oidium tabaci Thuem. in Contr. Mycol. Lusit., n. 218 (1879); Sacc., Syll. fungor., vol. IV, pag. 45. Su foglie di Nicotiana tabacum L. coltivata, Mohamed Aly, presso Addis Abeba, ca. 2400 m.s.m., 2/1/1939.

Oidiopsis sicula Scalia in Agr. calabro-siculo, anno XXVII, numero 24, pagg. 3—7 (1903) e in Rend. Congr. Bot. Palermo del 1902 (1904); Sacc., Syll. fungor., vol. XVIII, pag. 507. Su foglie di *Tropaeolum* sp. coltivato, presso Gondar (Amara), ca. 1950 m.s.m., 28/2/1939.

Analogamente a quanto è stato notato da Canonaco [Lavori del R. Ist. Bot. Palermo, vol. VIII, pag. 45 (1937)], si è osservato come il fungo sembri non passare dai *Tropaeolum* alle piante vicine; ciò che indurrebbe a pensare a una stretta relazione biologica tra ospite e parassita. Si sono inoltre riscontrati frequentemente gli organi di moltiplicazione della mucedinea in condizioni di una certa aridità: il che, se è forse in contraddizione con le affermazioni di Schweitzer [Arch. voor Rubbercult. Nederl.-Indie, vol. XII, pagg. 323—342 (1938)], sembra concordare con le osservazioni di Salmon circa la natura xerofitica del fungo.

Periconia pycnospora Fresen. in Beitr., I, pag. 20 (1850); Sacc., Syll. fungor., vol. IV, pag. 271. Su foglie di Manihot Glaziovii Muell., Saddu nella valle del Baro (Galla e Sidama), ca., 1300 m.s.m., 15/12/1938.

Ipofilla, nelle aree necrotiche di *Sphaerella* sp.

Helminthosporium Miyakei Nisikado in Special Rept. Ohara Inst. agr. res., vol. IV, pag. 384 (1928). Su infiorescenze di Eragrostis Teff (Zucc.) Trott., Malcò, presso Gimma (Galla e Sidama), ca. 1800 m.s.m., 21/11/1938.

Helminthosporium nodulosum Sacc., in Syll. fungor., vol. VI, pag. 421; sub *H. Nodosum* Berk. (*nomen nudum*) in Grev., vol. III, pag. 102 (1875). Su foglie di Eleusine coracana (L.) Gaertn. coltivata, Jambo, sulla pista da Bedelle a Gore (Galla e Sidama), ca. 1800 m.s.m., 9/12/1938 [1]).

Cercospora bauhiniae Syd. in Ann. Mycol., vol. XII, pag. 202 (1914); Sacc., Syll. fungor., vol. XXV, pag. 878. Su foglie di Bauhinia reticulata DC., valle del Diddessa (Galla e Sidama), ca. 1600 m.s.m., 3/12/1938.

Della due *Cercosporae* assai vicine segnalate sul genere *Bauhinia*: *C. latimaculans* Wakef. e *C. Bauhiniae* Syd., la forma osservata è stata riferita alla seconda per i cespitoli anfigeni, non confluenti e per le dimensioni dei conidiofori. I conidii invece misurano circa μ 70—150 ⩔ 4—5 e sono 12—20 settati, mentre nel tipo, segnalato a Los Baños (Filippine) su *Bauhinia malabarica* Roxb. essi sarebbero 3—6 settati e misurerebbero μ 35—65 ⩔ 4—5. Date però le oscillazioni amplissime dei valori dimensionali in *Cercospora*, in dipendenza della condizioni ambientali [Welles in Science, N.S., vol. LIX, pagg. 216—218 (1924)], si è creduto di non poter attribuire valore tassonomico alle differenze notate.

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[1]) Per l'esatta denominazione del patogeno vedi in E. Castellani (Agric. Colon., anno XXXII, no. 10 (1938)).

Cerebella andropogonis Ces. in Botan. Zeit., vol. IX, pag. 669 (1851); Sacc., Syll. fungor., vol. VII, pag. 523. Su infiorescenze di Hyparrhenia rufa Stapf (= Andropogon rufus Kunth.), vicino a Gimma (Galla e Sidama), ca. 1700 m.s.m., 19/11/1938.

Cerebella paspali Cooke e Massee in Grev., vol. XVI, pag. 20 (1887); Sacc., Syll. fungor., vol. VII, pag. 524. Su infiorescenze di Cenchrus sp., presso il lago Zuai (Galla e Sidama), ca. 1750 m.s.m., 1/2/1939.

I glomeruli, di forma quadrangolare-ovoidea (μ 18—22 ⩔ 16—18), di 2—7 cellule (μ 7—8 ⩔ 10—14), presentano negli esemplari più vecchi parete fumoso-olivacea, esternamente screpolata, come tassellata. A determinare questo particolare aspetto si crede però che molto abbia influito, oltre all' età, lo stato di conservazioni dei conidi.

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Summary.

The author presents the list of the fungus species gathered in Scioa country, in Amara and chiefly in the countries of Galles and Sidamo (East-Africa).

Characteristics of yeast causing clouding of dry white wines.

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Introduction.

Under the conditions which prevail during the storage of bottled dry white wines, at the winery as well as in retail stores, a cloud and/or sediment may develop. The formation of this cloud and/or sediment has been attributed in most cases to metallic contamination, chiefly iron and copper. In addition to copper and iron, MARSH (1940) states that tin, chromium, nickel and aluminum have been shown capable of causing clouding in wine. It has been recognized for many years that iron and copper cause a type of clouding in wine, termed "casse". The factors which characterize specific casses of these metals have been fairly well established.

Table 1, which is reproduced in part from MARSH (1940) describes the defects which characterize clouding of wine by iron or copper.

TABLE 1. *Characteristics of Iron and Copper Casse (Modified from Marsh,* 1940)

	Iron casse white	Iron casse blue	Copper casse
Kind of turbidity	Ferriphosphate	Ferritannate	Cuprosulfite
Appearance	Bluish-white opalescent haze finally depositing a white precipitate	Blue haze turning black upon flocculation and precipitation	Whitish haze turning brown upon flocculation
With access of air	Haze appears	Haze appears	Haze disappears

Another type of spoilage involving the clouding of wine is that resulting from the growth of lactic acid bacteria. This spoilage termed "tourne" in French literature, exhibits a peculiar silky sheen when the wine is gently agitated in the bottle. CRUESS (1935) and DOUGLAS and CRUESS (1936) investigated this spiolage and attributed it to *Lactobacillus Hilgardii:* FORNACHON et al. (1949) and Vaughn et al (1949) gave careful taxonomic consideration to the organisms involved and defined two species; namely *Lactobacillus hilgardii* and *Lactobacillus trichodes.* These organisms are quite susceptible to SO_2 (75 p.p.m.) and therefore easily controlled.

Still another type of cloud or sediment has recently appeared in wines of all classes, but most commonly in dry white wines. The formation of this cloud can first be detected by a fine yellowish-gray sediment, which upon shaking disperses as a cloud in the wine. In the later stages a permanent cloud forms and the sediment becomes brownish in color. This particular type of clouding in dry white wines was first observed in California bulk wine in 1936. The wine had been sulfured and subjected to the usual cellar treatment. The further addition of sulfur dioxide (approximately 25—50 p.p.m.) to the cloudy wine was followed by filtration, a procedure which appeared to be an effective means of control.

The following season a similar clouding appeared in bottled wine, and was treated in the manner indicated above. The addition of large dosages of sulfur dioxide solved the problem, though only temporarily. Since then, this type of spoilage has occurred to a limited extent each season in wines from most sections of California.

In so far as can be determined the occurrence of this new type of cloud in bottled wines appears to be limited to California. The first published reports on yeast clouding or sediment formation in bottled wines in California were those of BAKER (1936) and BAKER and CRUESS (1937) concerning sweet Sauterne; and that of PHAFF and DOUGLAS (1944) relating to fortified wines. Baker stated that on microscopic examination it was found that "in practically every case the cloudiness was due to growth of yeast". He succeeded in isolating several cultures of yeast from several samples of clouded Sauterne type wine. Taxonomy of these cultures was not included, designation being made only by the symbols S—2, S—3, S—5 and S—6. Culture S—2 was characterized as follows: cells oval to sausage shape, film formation, alcohol formation and fermentation negative, utilization of alcohol positive, growth in 16 % alcohol, and spores formed on carrot wedges. Although the type of media used and the spore shape observed were not mentioned, it appears that this organism was a member of the genus *Pichia*. Cultures S—3, S—5 and S—6 were characterized by Baker as follows: Cells predominantly round or sometimes oval, several sugars fermented with the formation of gas, 11 % alcohol produced in 20° Balling grape juice, spore formation negative on carrot and potato wedges. Culture S—3 grew in 13 % alcohol. These data are not sufficient to speculate on the taxonomy of these organisms. It is presumed that BAKER and CRUESS were able to reproduce clouding by inoculation into wine, since they suggest a control procedure based on laboratory tests. Pasteurization of bottled Sauterne at 60° C. for 30 minutes was suggested for one-fifth gallon and pint bottles, and 40 to 45 minutes at the same temperature for one-half and one gallon bottles.

PHAFF and DOUGLAS isolated the single species *Zygosaccharomyces mellis* from dessert wines. They were, however, unable to induce this organism to grow and produce a sediment in fortified wines.

According to FESSLER (1948) several methods of control, other than pasteurization, that have been tried in the industry are: use of sodium benzoate, monochloracetic acid, high concentrations of SO_2, and filtration through germ-proof filters.

It appears that the causitive organisms have gradually developed such a high tolerance to SO_2 (300—350 p.p.m.) that the amounts required for control result in an objectionable odor and flavor. The use of preservatives such as sodium benzoate and monochloracetic acid is considered unwise because of restrictions or complete prohibition by the Food and Drug laws.

The use of germ-proof filters as a means of control has failed because of the presence of large numbers of yeast cells in the wine and the occurrence of flaws in the filter pads. This combination of factors has been sufficient to enable a number of cells to pass through the filter and reinfect the wine.

Although the new type of cloud or sediment does not impart any off flavors or odors to the wine, it is detrimental to the appearance and hence reduces consumer acceptance. For this reason, and the fact that growth can occur over such a wide and interesting range of environmental conditions, a study of the characteristics of the yeasts responsible has been undertaken.

Experimental Procedures

Eighteen samples of clouded white wines were obtained from various wine production regions in California. All samples contained sediment and showed appreciable clouding, the characteristics of which did not conform to those caused by metallic contamination or *Lactobacillus Hilgardii.* Typical analyses of the clouded wine samples are given in Table 2.

TABLE 2. *Description of Samples*

Sample number	Origin of sample	Wine type	Year	SO_2 p.p.m.	Sugar gms/100 ml.	% alcohol by volume
3	Napa Valley	Sauterne	1946	165	.15	12.5
11	Santa Clara Valley	Sauterne	1946	170	.20	12.5
12	Santa Clara Valley	Sauterne	1946	170	.18	12.3
13	Santa Clara Valley	Pinot Blanc	1946	180	.14	12.1
21	Livermore Valley	Semillon	1945	224	.20	12.8
22	Livermore Valley	Semillon	1945	211	.20	12.7
31	Santa Clara Valley	Sauterne	1946	140	.21	12.5
32	Santa Clara Valley	Sauterne	1946	160	.19	11.8

Isolation of organisms from the clouded wines offered some difficulty. It was found essential to allow the wine to stand until sediment formation took place. Then 30 ml of wine and sediment were withdrawn and centrifuged for about 10 minutes under aseptic conditions. The clear liquid was decanted, leaving about 5 ml of liquid and sediment in the centrifuge tube. Ten per cent wort agar plate streak cultures were made and purification carried out by successive platings. Fourteen cultures were isolated and identified. Eight of the cultures were studied more extensively with respect to certain of their physiological characteristics.

Taxonomic procedures followed were those of STELLING-DEKKER (1931) and DIDDENS and LODDER (1942). The vegetable sporulation medium of MRAK, *et al.* (1942) was also used.

Comparative rates of fermentation of eight of the cultures isolated and *S. cereviseae* var. *ellipsoideus* were made by inoculating 500 ml sterile samples of diluted grape concentrate (32° Balling) with 5 ml of actively fermenting culture. Fermentation was carried out at room temperature. Sugar losses were determined periodically by use of the Lane-Eynon procedure (see Association of Official Agricultural Chemists, Methods of Analysis, 1945) until fermentation was complete.

Alcohol was determined by the procedure of JOSLYN and AMERINE (1941). Alcohol production tests were made in 120 ml bottles containing 100 ml of grape concentrate diluted to 21, 32, 35, 40 or 50° Balling and sterilized by heat. Fermentations were conducted at room temperature. These same samples were used to determine total and volatile acid production. Analyses were made in accordance with the procedures described by JOSLYN and AMERINE (1941). Volatile acid was calculated as grams of acetic acid, total acid as grams of citric per 100 ml. of liquid.

Ability of the isolated yeasts to grow in wine was ascertained by inoculating pasteurized wine (containing about 100 p.p.m. of SO_2 and 11.8 per cent alcohol), storing at room temperature and making actual counts after 0, 1, 3, 5, 7 and 10 days.

To determine the rate of growth at various temperatures it was first necessary to ascertain the temperature range over which growth could occur. This was done by streaking 10 per cent wort agar slants and then incubating at temperatures at 3° intervals over a range of 1° C. to 45° C. Growth occurred in the range 10—37° C. Following this, tubes of a pasteurized wine containing 12 per cent alcohol, .22 per cent sugar and 140 p.p.m. of SO_2 were inoculated with one ml. of the respective yeast from a 10 ml. suspension of yeast in wine and samples were incubated at the various temperatures between 10 and 37° C. After 10 days growth was determined by direct cell count with a Neuberg counting chamber.

To determine the minimum quantity of sugar required for growth, wine containing .22 per cent sugar and 12 per cent alcohol was diluted with a 12 per cent alcohol solution to give sugar concentrations of .20, .15, .10, and .01 per cent. Samples of the 12 per cent alcohol solution were used as controls. All samples were pasteurized, cooled, inoculated and then held at room temperature. Growth was determined by turbidity or film formation.

For growth observations at higher concentration of sugar, grape concentrate was diluted to 32, 35, 40 and 50° Balling, 150 ml added to 180 ml bottles, sterilized, cooled, inoculated and incubated at room temperature. Growth was determined by gas and film formation.

To determine the maximum alcohol concentration at which growth would occur, wine containing 14 per cent alcohol, was fortified to 15, 16, 17, 18, and 19 per cent alcohol, pasteurized at 62.7—65.5° C. for 30 minutes, cooled, inoculated and incubated at room temperature. Growth was determined by gas or film formation.

Ability of the organisms to grow in the presence of SO_2 was determined by adding various amounts of this compound to different samples, inoculating, and observing for growth. Similar observations were made on samples of diluted grape concentrate ranging in SO_2 content from 0 to 400 p.p.m. Observations were made for gas and film formation.

Experimental Results

Taxonomy of Organisms Isolated: From the eighteen samples of wine which contained a cloud or sediment fourteen yeasts were isolated. Eleven of these organisms proved to be members of the genus *Saccharomyces*, two *Pichia*, and one *Candida*.

The representatives of the genus *Saccharomyces* insofar as could be determined by comparison with published descriptions were *S. Chevalieri* Guilliermond, (cultures 21 and 22), *S. carlsbergensis* var. *monacensis* (Hansen) Dekker, (culture 12), and *S. oviformis* Osterwalder, (cultures 11, 13, 25, 26, 27, 28).

The two cultures of *S. Chevalieri* Guilliermond were identical with the description by STELLING-DEKKER with a slight variation in cell size, giant colonies and slant cultures. The size as given by STELLING-DEKKER for this species is (4—6) × (4—8) μ; culture 21 measured (4.8—7) × (5.3—8.8) μ; culture 22 measured (4.4—7.5) × (5.3—7.8) μ. Although the slant cultures are somewhat duller and not as smooth, and the giant colonies differ slightly in color, they conform closely to the description by STELLING-DEKKER. The variations are not significant for any taxonomic differentiation. It can therefore be concluded that the two cultures isolated are *Saccharomyces Chevalieri* Guilliermond.

Two cultures (21 and 22) were included in the species *S. Chevalieri* rather than the var. *torulosus* because of their large cell size. Inclusion in the var. *lindneri* was eliminated because this organism produces a slight growth in alcohol medium, whereas cultures isolated from wine did not. Ability to ferment raffinose $^1/_3$, excluded the wine cultures from the species *S. betacus* as recently described by MARCILLA, ALAS, and FEDUCHY (1936). Yeasts 2 and 4 of HOHL and CRUESS (1939) were similar to our cultures 21 and 22 except for cell shape and growth in alcohol media. HOHL and CRUESS gave the following cell measurements (3.1—6.2) × (3.9—9.2) μ for their culture 2. Culture number 4 of HOHL and CRUESS differed in that it produced moderate growth in alcohol. When our cultures 21 and 22 were grown in grape must a film was produced in approximately 8 days after completion of alcoholic fermentation. When inoculated in wine of 15.5 per cent alcohol a film was produced, completely covering the surface (4 oz. bottle), in 5 days. These characteristics as well as those of morphology conform to the characteristics of certain of the yeasts described by SCHANDERL (1936) and MARCILLA, *et al.* (1936), which are considered to be important in the production of Spanish Sherry.

Experiments conducted by PERELLI-MINETTI (1948) with a number of Spanish Sherry yeasts and culture number 21 of the authors, show that growth and film formation of the latter are quite similar to that of Jerez yeast number 5 of HOHL and CRUESS (1939), although the Sherry making ability of this culture has not yet been ascertained.

The isolate of *Saccharomyces carlsbergensis* var. *monacensis* (Hansen) Dekker (culture 12) agrees with the description given by STELLING-DEKKER with the exception of the size of the cells of our culture which were slightly smaller.

This organism was originally isolated by HANSEN from bottom beer yeast. Much later MRAK, *et al.* (1942) isolated a culture in California from soured figs. Although several other varieties of *S. carlsbergensis* have been isolated from grapes and grape products [var. *mandshuricus* by CASTELLI (1939) and var. *torulosus, valdensis, mandshuricus* and race *montibensis* by GAROGLIO (1941)] as far as can be ascertained *S. carlsbergensis* var. *monacensis* has not been previously isolated from either grapes or wine.

It is of interest that WILES (1949A) recently isolated as the causative agent for the cloud of bulk beer a strain of *S. carlsbergensis*, specifically termed Yorkshire Haze Strain I, since it differed somewhat from the five varieties listed by STELLING-DEKKER. WILES (1949B) also isolated from cloudy bottled beer two strains of *S. carlsbergensis*, one possessing characteristics of both *S. carlsbergensis* and *S. uvarum*, 10 of *S. cerevisiae*, 2 *Mycoderma* (possibly *Pichia* of *Hansenula*) and an asporogenus organism capable of forming a pseudomycelium.

The six isolates of *Saccharomyces oviformis* Osterwalder agree with the description by STELLING-DEKKER. Cells measured on 10 per cent wort agar after 10 days were found to be very large; (3.5—9.6) × (4.—12.2) μ; average, (6.0—8.7) μ. However, STELLING-DEKKER did not record cell measurements under these conditions. *Saccharomyces bayanus* Saccardo was eliminated as a possibility for these cultures because of the occurrence of long oval cells in young wort cultures; (3—6) × (8—18) μ. Cell measurements of one isolate were (3.9—5.7) × (4.4—6.6) μ; average, (5.2 × 5.7) μ.

Two cultures designated as *Saccharomyces cerevisiae* Hansen were quite similar to the Rasse Saaz (Obergärig) described by STELLING-DEKKER.

Representatives of the genus *Pichia* (cultures 31 and 32) were identified as *Pichia alcoholophila* Klöcker, and but for a few minor exceptions in giant colony and cell size, conformed to the description by STELLING-DEKKER.

The cell size as given by STELLING-DEKKER is (2—5) × (3.5—12) μ; cells of our culture 31 were (3.5—6.0) × (4.8—9.2) μ; average, (4.5 × 7.3) μ; and culture 32, (3.5—4.5) × (5.3—10.5) μ; average, (3.8 × 8.0) μ. The giant colony as described by STELLING-DEKKER indicates the presence of a mound in the center; cultures 31 and 32 differed by having a flat surface.

This species of *Pichia* was previously isolated from grapes and grape products in California by MRAK and MCCLUNG (1940) and in Italy by GAROGLIO (1941). CASTELLI (1939) isolated several other species in musts, among those being *P. membraefaciens*, *P. derossii*, and *P. chodati* var. *trumpyi*.

The single species of the genus *Candida* (culture 31) was identified as *Candida rugosa* (Anderson) Diddens and Lodder, and agreed in all respects with the description by DIDDENS and LODDER (1942). MRAK and MCCLUNG (1940) obtained several cultures of *Candida* and stated that species of this genus occur commonly on grapes. They also isolated 2 cultures of *Candida* from cloudy wines.

Fermentation Rates: The relative fermentation ability of five of the fermenting yeasts (cultures 11, 12, 13, 21, and 22) was compared with that of a Champagne variety of *S. cerevisiae* var. *ellipsoideus.*

In figure 1 is shown the grams of sugar utilized as plotted against the time in days. The original sugar content of the grape juice medium was 32.2 per cent. The rate of sugar conversion by *S. chevalieri* (cultures 21 and 22) and *S. carlsbergensis var. monacensis* (culture 12) was found to be approximately 1.5 times faster than that of *S. cerevisiae* var. *ellipsoideus.* They were also able to convert a total of 5.4 grams more sugar than the control culture (*S. cerevisiae* var. *ellipsoideus*). *S. oviformis* (cultures 11 and 13) utilized sugar at approximately the same rate as *S. cerevisiae* var. *ellipsoideus*, but the total amount utilized was greater by about 5.5 grams. In Table 3 is shown the total sugar utilized and the total alcohol produced by the five isolates and *S. cerevisiae* var. *ellipsoideus* from 32° Balling grape concentrate.

Alcohol Production: Alcohol production was also determined for the five yeasts. The yields obtained in the various concentrations of must is shown in Table 4. Maximum alcohol production of over 18 per cent by the fermenting isolates was obtained when a 32° Balling must, was used. When the Balling degree of the must was increased to 35° to 40° however, the amount of alcohol formed was decreased. This conforms with the experiments of Hohl (1938), working with *S. cerevisiae* var. *ellipsoideus*.

The observed maximum alcohol production was considerably higher than that usually reported. Sannino (1925) gave the usual maximum for wine yeast as 15 per cent, occasionally 16—16.5 per cent, and very rarely 17 per cent, Cruess, Brown and Flossfeder (1916) reported that in one experiment with syruped fermentations, (the addition of grape concentrate or raisins to the fermenting must) they were able to exceed 20 per cent by volume. Hohl and Cruess (1939) by straight fermentations of 30° Balling must, with Chalon and Jerez yeast, reported maximum yields of 18,0 and 17.7 per cent alcohol by volume, respectively. These data indicate that yields of 18 per cent alcohol and above are unusual in straight fermentations, but do not imply that high yields, as were obtained, are improbable.

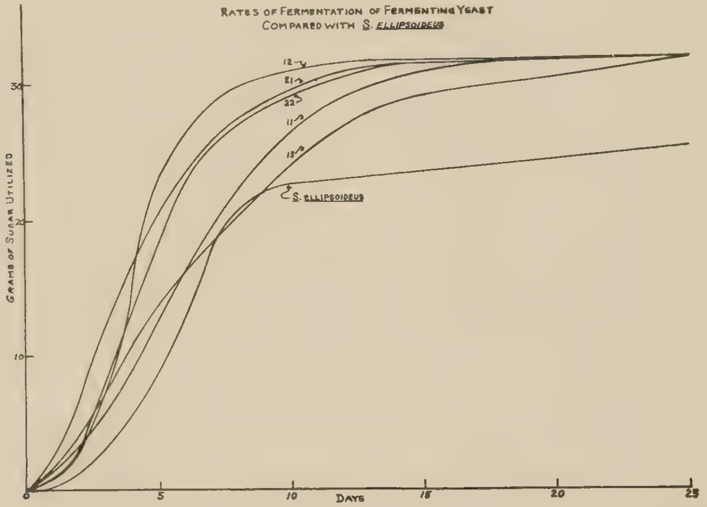

Figure 1. Grams of sugar utilized as plotted against the time in days.

Table 3. *Sugar Utilized and Alcohol Produced. From* 32° *Balling Must.*

	Culture	Sugar utilized (grams)	Alcohol Produced (by volume)
S. oviformis	11	31.87	18.45
S. carlsbergensis var. *monacensis*	12	31.81	18.15
S. oviformis	13	31.85	18.60
S. chevalieri	21	31.76	18.05
S. chevalieri	22	31.76	18.30
S. cerevisiae var. *ellipsoideus* . .		26.41	15.21

Acid Production: At the end of fermentation the samples used for alcohol production were analyzed for volatile and total acid content. In Table 5 is shown the volatile and total acid produced by the eight yeasts in grape juice of 21° Balling and in grape concentrate diluted to 32° Balling. It was observed that the highest amount of volatile acid was produced by *S. carlsvergensis* var. *Monacensis* (culture 12) (.089 grams of acetic acid per 100 ml.). *Pichia alcoholophila* produced only insignificant amounts. The volatile acid formation, of the eight cultures studied in 21° Balling must, was found to be well within the limitset by the California State Board of Health for wines (.11—.12 grams per 100 ml.); however, that produced in 32° Balling grape concentrate exceeded the limits from .05 to .10 grams per 100 ml. The total acid which was high in both musts was probably due to the high acid content of the juice used, and the fact that detartration was not carried out after fermentation.

TABLE 4. *Alcohol Production from Various Concentrations of Must*

	Culture	Per cent Alcohol by Volume			
		21° B.[1]	32° B.	35° B.	40° B.
S. oviformis	11	12.0	18.5	18.0	16.7
S. carlsbergensis var. *monacensis*	12	11.5	18.2	17.4	17.1
S. oviformis	13	12.3	18.6	18.2	17.2
S, chevalieri	21	11.4	18.1	17.8	16.5
S. chevalieri	22	11.7	18.3	18.1	16.8

1) Balling degree.

TABLE 5. *Volatile and Total Acid Production.*

	Culture	21° Balling		32° Balling	
		Volatile acid 1)	Total acid 2)	Volatile acid 1)	Total acid 2)
C. rugosa	3	.046	.45	.141	.60
S. oviformis	11	.076	.46	.210	.75
S. carlsbergensis var. *monacensis*	12	.089	.51	.207	.74
S. oviformis	13	.083	.49	.224	.77
S. chevalieri	21	.014	.44	.157	.70
S. chevalieri	22	.019	.41	.155	.69
P. alcoholophila	31	.010	.36	.090	.59
P. alcoholophila	32	.015	.42	.093	.64
Check sample		.010	.39	.033	.56

1) grams of acetic acid per 100 ml. of wine. 2) grams of tartaric acid per 100 ml.

From the results obtained it can be stated that in musts of normal sugar concentration (21—23° Balling) only small amounts of volatile acid are produced by these yeasts. In musts of higher concentrations (32° Balling and above) however, much greater amounts of volatile acid may be produced.

Growth in Wine: If these organisms could be made to grow directly in a natural wine, results obtained would be more significant, because natural spoilage conditions would be reproduced, and subsequent modifications of sugar, alcohol, and SO_2 concentrations could be made in this medium. This was readily accomplished by using a large inoculum.

In Table 6 is shown the relative increase in cell counts over a period of ten days. After five days a two-fold increase in cell count was evident. After ten days the two cultures of *S. chevalieri* (21 and 22) and the two cultures of *P. alcoholphila* (31 and 32) showed evidence of film formation. Two isolates of *S. oviformis* (11 and 13) and *S. carlsbergensis* var. *monacensis* (12) produced little sediment and no cloud in ten days, although a three-fold increase in cell count was obtained.

Under laboratory conditions it has been shown the organisms isolated from wine are capable of producing a cloud and sediment in reinfected wine. This is not proof of its wide spread occurrence in the wine industry, as there are other factors which can also be responsible for cloud and/or sediment formation, namely, metal and bacterial contamination.

Growth Rate at Various Temperatures: Determination of the growth rate at various temperatures is important because of the wide range of temperatures to which bottled wines are subjected during cellar and shelf storage.

TABLE 6. *Increase in Cell Count in Dry Wine. (Cells per ml.* × 10^5)

	Culture	Days					
		0	1	3	5	7	10
C. rugosa	3	2.4	3.2	4.8	7.7	7.9	8.8
S. oviformis	11	1.6	2.6	3.5	9.9	8.1	12.9 1)
S. carlsbergensis var. *monacensis*	12	2.0	2.0	3.0	5.2	7.2	26.9 1)
S. oviformis	13	2.4	3.8	4.1	11.3	12.1	22.5 1)
S. chevalieri	21	0.8	1.4	1.7	1.8	2.9	29.5 2)
S. chevalieri	22	1.0	1.4	1.8	2.3	6.3	12.4 2)
P. alcoholophila	31	1.8	2.8	3.2	3.2	4.2	4.9 2)
P. alcoholophila	32	1.2	1.2	2.4	3.0	4.0	4.8 2)

1) heavy sediment. 2) film formation.

In wine, a relatively lower optimum was indicated for all cultures (16—22° C.). *C. rugosa* (culture 3) gave greatest growth at 19° C. and had a very narrow range, not more than ± 2° C. The lowest optimum temperature recorded was for *S. oviformis* (cultures 11 and 13), at 16—19° C. *S. carlsbergensis* var. *monacensis* (culture 12) produced good growth from 19—31° C., with optimum growth at 22° C. A heavy film and sediment was obtained at temperatures of 19—31° C. for *S. Chevalieri* (culture 21 and 22) and *P. alcoholophila* (culture 31). The optima being 22 and 19° C. respectively. *P. alcoholophila* (culture 32) also produced a heavy film and sediment, but no growth was obtained at 31° C. Its optimum temperature was 19° C.

Table 7 shows the increase in cell number of the various cultures at specific temperatures. It is apparent that most intensive growth occurred at temperatures ranging from 16 to 22° C. This indicates that bottled wines are under the optimum temperature conditions for maximum growth to occur during shelf storage. Cellar storage, where temperatures lower than 16° C. prevail, would reduce the rate of growth but not inhibit it entirely.

The optimum range of 16—22° C. determined for the eight cultures, agrees closely with the observations of HOHL and CRUESS (1939) for film forming yeast.

TABLE 7. *Relative Increase in Cell Counts at Specific Temperatures in Wine* [1]) (*cells per ml.* × 10^5)

	Culture	Temperature (Degrees C.)									
		10	13	16	19	22	25	28	31	34	37
C. rugosa	3	1	1.5	1.6	6	2	1.3	1.2	1.2	1.2	1.2
S. oviformis	11	7	10	33	25	13	11	8	10	3	1.1
S. carlsbergensis var. *monacensis*	12	3	7	10	14	31	27	25	24	3.5	1.7
S. oviformis	13	7	6	19	22	10	8	8	8	8	1.3
S. chevalieri	21	0	2	5	47	50	45	45	42	13	2
S. chevalieri	22	1.8	7	.7	26	53	31	36	40	0	3.3
P. alcoholophila	31	2	4	5	25	22	22	23	21	0	0
P. alcoholophila	32	2	6	5	34	20	23	27	0	0	0

[1]) initial count = 1 × 10^5 cells per c.c.

Growth in Various Concentrations of Sugar: Reducing the sugar content of wines to .01 per cent failed to retard or decrease growth except in the case of *C. rugosa.* (See Table 8). As this is considerably below the minimum sugar content of dry wine, it was not considered necessary to conduct tests at concentrations below .01 per cent sugar. The occurrence of growth at these low concentrations of sugar indicates that wines with low residual sugar content are not necessarily protected from deterioration by the yeast cloud organisms. Films were formed by *S. chevalieri* and *P. alcoholophila* on all samples, ranging

TABLE 8. *Growth at Low Sugar Concentrations in Wine*

	Culture	Sugar Content (per cent)					
		.25	.20	.15	.10	.05	.01
C. rugosa	3	+	+	+	—	—	—
S. oviformis	11	+	+	+	+	+	+
S. carlsbergensis var. *monacensis* . .	12	+	+	+	+	+	+
S. oviformis	13	+	+	+	+	+	+
S. chevalieri	21	+	+	+	+	+	+
S. chevalieri	22	+	+	+	+	+	+
P. alcoholophila . . .	31	+	+	+	+	+	+
P. alcoholophila . . .	32	+	+	+	+	+	+
Check		—	—	—	—	—	—

in sugar concentration from .25 to .01 per cent. Heavy sediments were formed by *S. oviformis* and *S. carlsbergensis* var. *monacensis* at these concentrations. All eight yeasts isolated grew at 40° Balling but none at 50° Balling.

Growth in Wine with Various Concentrations of Alcohol: Eight cultures were found capable of growing in wine containing 13 per cent alcohol, and all but *C. rugosa* grew in wine with 14 per cent. *P. alcoholophila* grew in 14 but not 15 per cent; *S. Chevalieri* grew in 16 but not 17 per cent, and cultures of *S. oviformis* and *S. carlsbergensis* var. *monacensis* showed but slight growth at 17 per cent and none in 18 per cent (See Table 9). Film formation of *S. chevalieri* was very slight in 16 per cent alcohol. The latter agrees with the results obtained by HOHL and CRUESS (1939) for Spanish Sherry yeast.

TABLE 9. *Growth at Various Alcohol Concentrations*

	Culture	Per cent Alcohol in Wine [1]					
		13	14	15	16	17	18
C. rugosa	3	+	—	—	—	—	—
S. oviformis	11	+	+	+	+	±	—
S. carlsbergensis var. *monacensis* . .	12	+	+	+	+	±	—
S. oviformis	13	+	+	+	+	±	—
S. chevalieri	21	+	+	+	±	—	—
S. chevalieri	22	+	+	+	±	—	—
P. alcoholophila . . .	31	+	+	—	—	—	—
P. alcoholophila . . .	32	+	+	—	—	—	—

[1] wine contains .22 % sugar.)

Growth in Wine With Various Concentrations of SO_2: High concentrations of SO_2 were found necessary to inhibit growth completely (300—350 p.p.m.). All cultures except *C. rugosa* grew at 250 p.p.m. *P. alcoholophila* (cultures 31 and 32) was inhibited at 300 p.p.m. and only slight growth was obtained by the other cultures at this concentration. All cultures were inhibited by 350 p.p.m. SO_2 (See Table 10).

TABLE 10. *Growth in Dry Wine Containing Various Concentrations of* SO_2

	Culture	p.p.m. SO_2			
		200	250	300	350
C. rugosa	3	+	—	—	—
S. oviformis	11	+	+	±	—
S. carlsbergensis var. *monacensis*	12	+	+	±	—
S. oviformis	13	+	+	±	—
S. chevalieri	21	+	+	±	—
S. chevalieri	22	+	+	+	—
P. alcoholophila	31	+	+	—	—
P. alcoholophila	32	+	+	—	—

The use of maximum concentrations of SO_2 allowed by Federal and State restrictions (350 p.p.m.), according to AMERINE and JOSLYN (1940), appear necessary to prevent the growth of these organisms in wine. According to CRUESS (1948) the practice in many wineries, during the past few seasons, has been to add as much as 400 p.p.m. of SO_2 to the wine at bottling, with the assumption that during the storage before sale the SO_2 content will decrease sufficiently to be within the permissable maximum limit by the time the finished product reaches the jobber. Whether or not this method of preservation is effective has not yes been ascertained.

TABLE 11. *Growth in 21° Balling Must Containing Various Concentrations of* SO_2

	Culture	p.p.m. SO_2				
		200	250	300	350	400
C. rugosa	3	+	+	—	—	—
S. oviformis	11	+	+	+	—	—
S. carlsbergensis var. *monacensis*	12	+	+	—	—	—
S. oviformis	13	+	+	+	—	—
S. chevalieri	21	+	—	—	—	—
S. chevalieri	22	+	—	—	—	—
P. alcoholophila	31	+	+	+	+	—
P. alcoholophila	32	+	+	+	+	—

Growth in Must Containing Various Concentrations of SO_2: Organisms were inoculated into sterile samples of 21° Balling must to which various quantities of SO_2 were added. All cultures grew in must containing 200 p.p.m. SO_2. Cultures of *S. chevalieri* were inhibited by 250 p.p.m.; *S. carlsbergensis* var. *monacensis* and *C. rugosa* by 300 p.p.m.; *S. oviformis* by 350 p.p.m.; and *P. alcoholophila* by 400 p.p.m. (see Table 11).

The use of high SO_2 concentrations such as were found necessary to inhibit growth in must, would be impracticable since the desired wine yeasts would also be inhibited at these concentrations.

The results obtained are contrary to the work of CRUESS (1912) which showed that 100 p.p.m. SO_2 would eliminate 99.9 per cent of the wild yeast, mold, and bacterial cells in a normal must. It appears that the cultures isolated have gradually increased their SO_2 tolerance, just as PORCHET (1931) showed was the case for true wine yeasts, or more likely that CRUESS' isolates did not include any of the yeasts isolated by the present authors from wines.

Summary

Fourteen cultures of yeast were isolated from dry white wines which contained a cloud and/or sediment. These organisms were identified as *S. chevalieri* Guilliermond (2 cultures), *S. carlsbergensis* var. *monacensis* (Hansen) Dekker (1), *S. oviformis* Osterwalder (6), *S. cerevisiae* (2), *Pichia alcoholophila* Klöcker (2), and *Candida rugosa* (Anderson) Diddens and Lodder (1).

Various characteristics of these organisms were investigated. These included, in addition to morphology and taxonomy for all cultures; fermentation rates, alcohol production, acid production, growth in wine, optimum temperatures, minimum and maximum sugar tolerance, alcohol tolerance in wine, sulphur dioxide tolerance in wine, and sulphur dioxide tolerance in grape must for eight of the cultures.

Observation by various investigators indicate that the organisms isolated occur commonly in the wine industry and are therefore not rare or unexpected species.

A series of laboratory tests show that these organisms can cause a cloud and/or sediment in wine.

Two isolates termed *S. chevalieri* were of particular interest since they possessed characteristics very similar to those of Spanish Sherry yeasts as characterized by SCHANDERL (1936), MARCILLA, et al. (1936) and HOHL and CRUESS (1939).

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Estudio y clasificacion de 156 cepas del genero „Candida" Berkhout, 1923. - Consideraciones sobre candidomicosis, (Moniliasis).-[1]

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Introducción:

Cuenta la Micoteca de éste Instituto de Clínica Quirúrgica con un número cercano a quinientas cepas de hongos, siendo la mayoría desorígen humano. Han siodo aisladas de materiales patológicos y normales. Otras cepas se han solicitado a distintos Centros Micológicos y han servido de estudio y comparación para las primeras. Finalmente, algunas han side enviadas de países vecinos para su identificación.

En 1945, dos de los autores publicaron un primer trabajo (13), determinando el género de los honges levaduriformes que tenía la Micoteca en ese entonces. En 1949, en éste trabajo, los autores clasifican el total de cepas del género *Candida* que actualmente tiene la Micoteca. Dejan constancia que han seguido el cuadro de clasificación propuesto per les autores uruguayos Mackinnon y Artagaveytia-Allende (11), y considerado les trabajos de Langeron y Talice (9); Langeron y Guerra (10); Diddens y Lodder (5), y Mackinnon (12).

Se han agrupado las cepas según su orígen. Al final de cada una de éstas agrupaciones se hacen algunas consideraciones clínicas.

Cepas de orígen bronco-pleuro-pulmonar:

Se estudiaron 52 cepas, todas ellas aisladas de esputos, menos 2 que fuéron de orígen pleural. Se hace el diagnóstico de Candidomicosis solamente en aquelles casos que cumplieron les antecedentes necesarios para establecer dicho diagnóstico. Varias cepas fuéron aisladas como gérmenes de asociación del verdadero agente etiológico, haciéndose notar en cada caso. El número que se anota en la primera columna corresponde al registro de la Micoteca del Institute de Clínica Quirúrgica.

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[1] Trabajo presentado a la 4a. Jornada Clínica de Verano en el Hospital Viña del Mar los días 25-26-27-28 de Enero de 1950. (Viña del Mar; Chile).

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55	— Cepa 267 L.P.F.M., París	*Candida tropicalis*
58	— Cepa Magallanes. Candidomicosis bronquial	*Candida albicans*
68	— *Mycotorula alvarezsotoi* Mazza-Niño, 1930. Candidomicosis generalizada	*Candida albicans*
71	— Misión Universitaria No. 31348	*Candida albacans*
74	— R.G. 57765. Candidomicosis bronquial	*Candida tropicalis*
75	— R.G. 60247. Candidomicosis bronquial	*Candida albicans*
83	— Cepa San Miguel. Candidomicosis bronquial	*Candida krusei*
84	— Cepa Latorre. Candidomicosis bronquial	*Candida tropicalis*
87	— R.G. 67.186. Asociada a Granuloma criptococcico	*Candida albicans*
90	— M.U. 36.391. Asociada a Granul. paracoccidioíd	*Candida albicans*
106	— Cepa Piñero	*Candida albicans*
113	— R.G. 67.186. Asociada a Granuloma criptococcico	*Candida albicans*
116	— Cepa Méndez. Candidomicosis bronquial	*Candida albicans*
117	— Cepa Mitidiero. Candidomicosis bronquial	*Candida albicans*
120	— I.C. Esputes	*Candida albicans*
122	— Quiste hidatídico de pulmón	*Candida albicans*
123	— Bronquítis crónica hemoptoica	*Candida albicans*
126	— Absceso gangrenoso del pulmón	*Candida albicans*
128	— Cepa Varela	*Candida albicans*
129	— Cepa Varela	*Candida albicans*
130	— R.G. 74061	*Candida albicans*
150	— R.G. 84419. Abscesos múltiples de hígado	*Candida albicans*
156	— Cepa Olaeta. Candidomicosis bronquial	*Candida albicans*
165	— Lesion pulmon derecho tipo tumoral	*Candida albicans*
183	— Esputos hemoptoícos	*Candida albicans*
192	— R.G. 91391	*Candida albicans*
199	— R.G. 90956. Punción pleural	*Candida albicans*
229	— R.G. 103.761	*Candida albicans*
234	— R.G. 104.309. Asociada a Actinomicosis pulmón	*Candida albicans*
239	— Cepa Madrid. Asoc. a *Mycobacterium tuberculosis*	*Candida albicans*
242	— Cepa Górdon	*Candida albicans*
250	— Cepa Valenzuela	*Candida albicans*
251	— Cepa Valenzuela	*Candida albicans*
252	— Cepa Valenzuela	*Candida albicans*
255	— Cepa Alvarado	*Candida albicans*
256	— Cepa Valenzuela	*Candida albicans*
257	— Cepa Introzzi. Cancer del pulmón	*Candida albicans*
301	— Cepa Sra de Sperandre	*Candida albicans*
303	— R.G. 113.159. Absceso del pulmón	*Candida albicans*
324	— Cepa Benetti	*Candida albicans*
413	— Cepa Blanco	*Candida albicans*
423	— R.G. 129.787. Cepa A.	*Candida albicans*
423	— R.G. 129.787. Cepa B.	*Candida tropicalis*
426	— Cepa Lombrodo	*Candida krusei*
429	— R.G. 130.483	*Candida albicans*
433	— R.G. 96610	*Candida krusei*
435	— R.G. 134.591. Asoc. a *Mycobacterium tuberculosis*	*Candida albicans*
437	— R.G. 133.234	*Candida tropicalis*
444	— R.G. 135.113. Asoc. con Granuloma paracoccicioíd	*Candida albicans*
446	— Cepa Groisman. Asoc. a Grnauloma paracoccidioíd	*Candida albicans*
450	— Cepa Armayor. Bronquítis asmática	*Candida albicans*
454	— Cepa Pérez. Bronquítis crónica	*Candida albicans*

Resumiendo la identificación de las cepas de orígen bronco-pleuro-pulmonar, encontramos:

Candida albicans	44
Candida tropicalis	5
Candida krusei	3
Total de cepas	52

Consideraciones — Dos especies del género *Candida* Berkhout, 1923; prueban constantemente tener poder patógeno, en ciertas circunastancias, para el hombre, Ellas son *Candida albicans* (Ch. Robin, 1853) Berkhout, 1923; y *Candida tropicalis* (Castellani, 1910) Berkhout, 1923.

La virulencia de otras especies de éste género es probable. Muchos autores la afirman para algumas de ellas. Es posible que así sea, dependiendo acaso de la resistencia disminuída del organismo atacado. Lo corriente es encontrar las dos especies nombradas como habituales agentes etiológicos de la Candidomicosis.

Aislar alguna especie de éste género no significa afirmar su rol patógeno en la afección que se estudia. Puede corresponder a la flora asociada del verdadero agente, o bien tratarse de una simple contaminación de la muestra o del médio de cultivo.

Depende de la naturaleza del material estudiado el número de factores que se tendrá en cuenta para negar o afirmar su rol patógeno. Es corriente, por ejemplo, obtener examen directo y cultivo positivo de *Candida albicans* en el estudio microbiológico de una expectoración, No por ello se diagnostica Candidomicosis de las vías respiratorias. Primero deberá establecerse que dicho agente no se haya incorporado a la muestra durante el pasaje bucofaríngeo, practicando un buen examen bucal para eliminar dicha posibilidad. Luego se tratará de obtener cierto grado de limpieza bucofaringea antes de coleccionar la muestra. Se recurrirá en los casos dudosos a la extracción del material bronquial mediante el broncoscópio. Finalmente debe insistirse siempre en la búsqueda del *Mycobacterium tuberculosis* y otros conocidos agentes habituales de procesos broncopulmonares, antes de afirmar un diagnóstico de Candidomicosis broncopulmonar.

Se ha dado el ejemplo del anterior diagnóstico por ser más dificil de establecer, dado los factores de confusión ya enumerados. Con ello se quiere recomendar prudencia, pero en ningún caso desanimar.

Candidomicosis broncopulmonar — La existencia de esta entidad clínica es una verdad indiscutible. Este concepto se ha desacreditado por el abuso con que se ha afirmado y negado sin seriedad y estudio necesarios en la mayoría de los casos.

Muchas veces el hallazgo de alguna especie de *Candida*, en el estudio de una expectoración, sólo corresponde a un gérmen de asociación o contaminación, sin que pueda atribuírsele valor patógeno sino después de eliminar la serie de factores de confusión ya enumerados. Es frecuente encontrar *Candida albicans* asociada a procesos pulmonares como la Tuberculosis, neumonia, bronquiectasia, Granuloma paracoccidioídico, etc. En cambio *Candida tropicalis* corresponde con más frecuencia a Candidomicosis primitiva e independiente de otros procesos del árbol respiratorio.

La experiencia personal de los autores les hace afirmar la existencia de Candidomicosis broncopleuropulmonar. De qué otra manera puede explicarse la existéncia de formas clínicas en las cuales solo se encuentra como posible agente etiológico a una misma especie aislada en repetidas ocasiones, con ausencia de cualquier otro gérmen, y que luego evolucionan tán favorablemente curando con tratamiento yodado. Finalmente han podido presenciar la punción de empiemas pleurales que han dado salida a cultivos puros de *Candida albicans* (7).

Las formas clínicas pueden ir de la bronquítis crónica simple y bronquítis asmatiforme (3) hasta las formas graves broncopulmonares y pleurales. Se inicia con el cuadro clínico de una bronquítis, a veces febril, acompañada generalmente de abundante expectoración la que puede ser serosa, mucopurulenta, y hasta hemoptoica lo que puede hacer pensar en una Tuberculosis. Corrientemente no hay compromiso del estado general en las etapas iniciales, sin embargo, posteriormente puede acompañarse de perdida de peso y astenia aumentando así las posibilidades de confundirla con una Tuberculosis.

El estudio radiológico es de gran ayuda, pero el diagnóstico lo establece el microbiólogo. Primero debe excluirse los habituales agentes por medio de cultivos e inoculación experimental especialmente para el *Mycobacterium tuberculosis*. Después de esto podemos darle valor al hallazgo repetido de una misma especie de *Candida*. El diagnóstico indirecto por médio de intradermoreacciones y reacciones de fijación de complemento son de valor dudoso, pudiéndose intentar como factores auxiliares. No se han observado alteraciones constantes en la fórmula hematológica en éstos casos.

Bien establecido el diagnóstico se recurre al tratamiento yodurado. Puede hacerse con dosis crecientes de yoduro de potasio por vía oral y yoduro de sodio intravenoso, buscando la tolerancia del enfermo. La dosis terapéutica varía mucho de un enfermo a otro, y pareciera guardar relación con los límites de tolerancia individual. Lo importante, una vez establecida la dosis de tolerancia es mantenerla prolongadamente. Se ha usado con éxito la solución de Yatrén al 3 % en dosis de 2 c.c. inyectadas intravenosa diariamente junto con yoduros por vía oral. En los casos rebeldes se ha obtenido buen resultado con violeta de genciana inyectada intravenosamente en una solución al 5 por 1000 (cinco por mil) a una dosis diaria de cinco milígramos por kilo de peso, hasta completar de 4 a 6 dosis.

Cepas aisladas de boca y faringe:

49	— Cepa 104 I.P.F.M. París. Muguet	*Candida albicans*
53	— Muguet generalizado a todo el tubo gigestivo	*Candida albicans*
54	— Rinofaringítis	*Candida albicans*
57	— Glosítis. Cepa Alievi	*Candida albicans*
86	— Glosítis. Cepa Miyara	*Candida albicans*
82	— Muguet. Enfermo con cancer hepático	*Candida albicans*
91	— Leión labial. Asoc. a Granuloma paracoccidiodico	*Candida albicans*
101	— Glosítis. Cepa Quintere	*Candida albicans*
168	— Muguet. Cepa Walkers	*Candida albicans*
207	— Lesion dorso de longua en tuberculoso	*Candida albicans*
208	— Lesion dorso de longua en tuberculoso	*Candida albicans*
238	— R.G. 106.219. Cancer del maxilar	*Candida albicans*
240	— R.G. 107.823. Lengua nigra pilosa	*Candida albicans*
295	— Estomatítis. Cepa Marchesi	*Candida flareri*
302	— Ulceracienes en paladar blando. Enf. luético	*Candida albicans*
363	— Estomatítis. Cepa Urdizaga	*Candida pseudotropicalis*
405	— Lesion ulcerosa mucosa mejilla derecha	*Candida albicans*

Resumiendo la identificación de las cepas aisladas de boca y de faringe, se encuentra:

Candida albicans	15
Candida pseudotropicalis	1
Candida flareri	1
Total de cepas	17

Consideraciones — El agente etiológico habitual del Muguet es *Candida albicans*. ARTAGAVEYTIA-ALLENDE, en su interesante contribución al estudio de la microbiología bucal (1), encuentra *Candida albicans* aproximadamente en un 25 % en la cavidad bucal de personas sanas.

La patogenia del Muguet está condicionada por regla general a una alteración del estado inmunitario. Frecuente en la primera infancia, en niños cuyas defensas orgánicas están bajas por mala nutrición o complicación de otras enfermedades. Menos frecuente en el adulto, se presenta generalmente en terrenos debilitados, caquécticos, como ocurre en las etapas finales del cancer y la tuberculosis. Tambien trastornos humorales, como la diabetis, pueden favorecer su aparición. No siempre es manisfestación secundaria, pues suelen encontrarse casos primitivos, siendo en éstos menos extensa la alteración y correspondiendo a ulceraciones más limitadas de la cavidad bucofaríngea.

La sintomatología se inicia con una sensación de ardor que corresponde a una estomatítis eritematosa, con cierta tumefacción, sequedad y agrietamiento de la mucosa bucal, la que posteriormente se cubre de una capa cremosa blanquecina. Esta capa puede alcanzar varios milímetros de espesor, siemdo de fácil desprendimiento y dejando un fondo rojo edematoso muy sensible que dá al paciente la sensación de quemadura. Dificulta las funciomes de la masticación y deglución de los alimentos, agravando el cuadro clínico con ello.

El estudio histopatológico muestra congestión y edema de la mucosa con hiperplasia papilar y abundante descamación superficial. Entre éstos últimos elementos se encuentran abundantes formas levaduriformes y pseudofilamentosas que corresponden a *Candida albicans*. Estas formas pueden traspasar el cuerpo mucoso y llegar hasta los vasos del corion. Es fácil comprender que en éstas condiciones se absorban substancias que provoquen a distancia reacciones de tipo alérgico: paraqueratosis cutáneas, alopecía, etc. En éstos casos es posible una diseminación sanguínea.

El diagnóstico clínico es sencillo, pero lo es más el micológico. Basta con examinar al microscópio el exudado, ya sea al fresco o en frotis teñido con Gram, para comprobar la presencia de abundantes elementos levaduriformes y filamentosos. El cultivo se obtiene con facilidad, sembrando en el medio de Sabouraud o en simple agar glucosado. Crece bien a 37°, desarrollando también a temperatura ambiente.

El pronóstico del Muguet como afección primaria es benigno. Cuando es manifestación secundaria, como ocurre en la mayoría de los casos, su pronóstico depende de la evolución que siga la causa que favorece su desarrollo.

Como tratamiento local se recomiendan las tocaciones con una solución acuosa de violeta de genciana al $5\ ^0/_{00}$, varias veces al día. Lavados bucales con agua bicarbonatada destinados a combatir la acidez que favorece el desarrollo del hongo. Prima levantar el estado general con transfusiones sanguineas, aminoácidos y vitaminas.

Es frecuente que las placas de Muguet se extiendan hacia la faringe y puedan aún comprometer la mucosa esofágica y generalizarse al tubo digestivo. Fuera de ésta posibilidad se han encontrado localizaciones aisladas de Candidomicosis de las amigdalas y faringe.

Cepas de origen intestinal:

51	— Sprue. Blastodendrium intermedium Cif. y Ashf., 1929 . . .	*Candida intermedia*
111	— R.G. 71940. Cateterismo duodenal.	*Candida albicans*
209	— Deposiciones. Alérgia intestinal	*Candida albicans*
210	— Sondaje duodenal	*Candida krusei*
261	— R.G. 110.581. Lavado gástrico	*Candida albicans*
277	— Sprue. Monilia parapsilosis Ashford	*Candida parakrusei*
311	— R.G. 117.142. Bilis	*Candida krusei*
312	— R.G. 118.260. Bilis	*Candida albicans*
313	— Cepa Giagnoni. Bilis.	*Candida guilliermondii*
314	— R.G. 118.602. Bilis	*Candida parakrusei*
315	— R.G. 95994. Bilis	*Candida guilliermondii*
316	— Bilis B.	*Candida tropicalis*
325	— R.G. 120.031. Bilis	*Candida albicans*
338	— R.G. 121.500. Bilis A y B.	*Candida chalmersii*
339	— R.G. 121.917. Bilis B	*Candida flareri*

382 — Bilis	*Candida chalmersii*
386 — Cepa Ojeda. Bilis	*Candida chalmersii*
415 — R.G. 129.789. Bilis B.	*Candida albicans*
422 — Bilis	*Candida tropicalis*
447 — R.G. 136.150. Bilis	*Candida tropicalis*
457 — Raspado mucosa intestinal post-morten. Disent.	*Candida albicans*

Resumiendo la identificación de las cepas de orígen intestinal, aparece:

Candida albicans	8
Candida tropicalis	3
Candida chalmersii	3
Candida parakrusei	2
Candida krusei	2
Candida guilliermondii	2
Candida intermedia	1
Candida flareri	1
Total de cepas	22

La Candidomicosis intestinal puede producirse a consecuencia de una generalización de Muguet. Como entidad clínica independiente es muy discutida. Algunos autores han llegado a atribuirle relación con el Sprue. Posteriormente se ha negado esta posibilidad, demostrando la presencia de algunas especies de *Candida* en deposiciones normales y afirmando que ellas son ingeridas con los alimentos. En éste Laboratorio se han encontrado distintas especies en contenidos gástricos y duodenales. Existiendo en el intestino no sería extraño que en alteraciones digestivas o generales pudieran adquirir virulencia o exaltaran la de otros agentes patógenos del intestino.

Cepas de orígen génito-urinario:

191 — Pus absceso ovárico. Sala X-Ficha 10999	*Candida albicans*
213 — Flujo vaginal. Leurorrea R.G. 97737	*Candida albicans*
216 — Flujo vaginal. Leucorrea. R.G. 98812	*Candida albicans*
217 — Flujo vaginal. Leucorrea. R.G. 100.178	*Candida albicnas*
299 — Flujo vaginal. Enf. en gestaciòn avanzada	*Candida albicans*
336 — Flujo vaginal. R.G. 121.458	*Candida guilliermondii*
364 — Flujo vaginal. Leucorrea. R.G. 118.752	*Candida albicans*
385 — Flujo vaginal. Leucorrea. R.G. 125.441	*Candida albicans*
408 — Flujo vaginal. Leucorrea	*Candida albicans*
420 — Flujo vaginal. Leucorrea. R.G. 131.813	*Candida albicans*
439 — Flujo vaginal. Leucorrea	*Candida albicans*
443 — Flujo vaginal. Leucorrea	*Candida albicans*

Resumiendo la identificación de éste grupo de cepas se encuentra:

Candida albicans	11
Candida guilliermondii	1
Total de cepas	12

Consideraciones — La Candidomicosis del tráctus genital de la mujer es una entidad patológica que no se discute y el predomínio casi exclusivo de *Candida albicans* como agente etiológico queda confirmado una vez más, (2), (8).

A la leucorrea acompaña un proceso inflamatorio de vulvo-vaginítis con intenso prurito, muy molesto, que se constituye en la desesperación de las enfermas y es factor coadyubante para otras complicaciones. Con frecuencia puede coexistir con intértrigo inguinal blastomicético.

El diagnóstico micológico se efectúa con facilidad; para ello nos valemos del examen microscópico de preparados frescos, de frotis, coloreados con el Gram y de cultivos en agar glucosado de Sabouraud.

Hecho el diagnóstico de Candidomicosis se impone una terapéutica adecuada. Apósitos húmedos con una solución de biborato de sodio al 4 %; pincelaciones una vez por día con una solución de violeta de genciana al 2—4 % hacen desaparecer el prurito, el síntoma más molesto, alcanzando en poco tiempo la curación clínica y parasitaria.

Cepas aisladas de piel y anexos:

41	— Cepa Perna. Onixis y perionixis	*Candida albicans*
43	— Cepa Pizzo. Onixis y perionixis	*Candida albicans*
44	— Cepa Kros. Onixis y perionixis	*Candida albicans*
45	— Cepa Galli. Onixis y perionixis	*Candida albicans*
46	— Cepa Leite. Onixis y perionixis	*Candida albicans*
47	— Cepa Frassini. Onixis y perionixis	*Candida albicans*
48	— Cepa Zucinios. Onixis y perionixis	*Candida albicans*
52	— *Mycocandida onychophila*. Onixis	*Candida parakrusei*
56	— Onixis y perionixis	*Candida albicans*
59	— *Mycotorula periunguealis* Niño, 1929	*Candida albicans*
60	— R.G. 48490. Onixis y perionixis	*Candida albicans*
61	— R.G. 50071. Onixis y perionixis	*Candida albicans*
62	— *Mycocandida inexpectata*. Onixis y perionixis	*Candida krusei*
63	— R.G. 51122. Onixis y perionixis	*Candida albicans*
64	— R.G. 45559. Onixis y perionixis	*Candida albicans*
65	— Cepa Reuter. Onixis y perionixis	*Candida parakrusei*
66	— Cepa González. Onixis y perionixis.	*Candida albicans*
67	— R.G. 45995. Onixis y perionixis	*Candida parakrusei*
69	— R.G. 45909. Onixis y perionixis	*Candida tropicalis*
70	— Cepa Costa. Onixis y perionixis	*Candida albicans*
73	— Cepa Aurora. Onixis y perionixis	*Candida albicans*
76	— Cepa Vázquez. Intértrigo	*Candida albicans*
77	— Cepa Belzoni. Intértrigo inguinal.	*Candida albicans*
78	— R.G. 67186. Asoc. a absceso Granuloma criptococc.	*Candida albicans*
85	— Cepa Jörg. Intértrigo	*Candida albicans*
92	— R.G. 68382. Intétrigo genital	*Candida albicans*
104	— R.G. 70709. Onixis y perionixis	*Candida albicans*
119	— R.G. 54090. Onixis y perionixis	*Candida albicans*
121	— R.G. 72783. Onixis y perionixis	*Candida albicans*
145	— R.G. 79491. Onixis y perionixis	*Candida albicans*
148	— Cepa Serraco. Onixis y perionixis	*Candida albicans*
158	— Cepa Raggio. Intértrigo interdigital	*Candida albicans*
169	— R.G. 88092. Onixis y perionixis	*Candida albicans*
170	— R.G. 88180. Onixis y perionixis	*Candida albicans*
173	— R.G. 88992. Onixis y perionixis	*Candida albicans*
175	— Cepa Olivares. Onixis y perionixis	*Candida albicans*
194	— Cepa Linares. Dermatítis piel del abdomen	*Candida parakrusei*
201	— Cepa Bossio. Onixis y perionixis.	*Candida albicans*
205	— Onixis y perionixis	*Candida albicans*
224	— R.G. 101.520. Pus abscesos mama izquierda	*Candida chalmersii*
227	— Cepa Uriol. Costras querion cuero cabelludo	*Candida parakrusei*
236	— Cepa Hugo. Lesión eczematiforme del puño.	*Candida parakrusei*
294	— Cepa Suaya. Onixis y perionixis	*Candida albicans*
296	— Cepa Capdevila. Intértrigo sub-mamario	*Candida albicans*
304	— Intertrigo interdigital	*Candida chalmersii*
328	— R.G. 120.289. Intértrigo interdigital	*Candida albicans*
371	— Cepa Quito. (Prof. Luís Leóin). Ulcera de pierna	*Candida albicans*
440	— Cepa Muro. Intértrigo inguinal y genital	*Candida albicans*
441	— Cepa Russo. Intértrigo inguinal	*Candida albicans*
453	— Cepa Galache. Onixis y perionixis	*Candida albicans*

Resumiendo las cepas encontradas, tenemos:

Candida albicans	40
Candida parakrusei	6
Candida chalmersii	2
Candida krusei	1
Candida tropicalis	1
Total de cepas	50

Comprobamos también para las localizaciones en la piel y sus anexos el gran predomínio de *Candida albicans*, cuyo rol patógeno en los intértrigos húmedos y en las onixis y perionixis es evidente. La Candidomicosis localizada en las uñas y en el repliegue periungueal es afección frecuente de observar sobre todo en la mujer, como lo ha puntualizado uno de nosostros en numerosos trabajos (14). El intértrigo blastomicético interdigital, inguinal, del surco sub-mamario o de otros repliegues cutáneos, aunque de localización menos frecuente que la anterior, está lejos de ser rara, baste recordar que el llamado „Pié de atleta" es de ésta etiología en un alto porcentaje de casos.

El examen microscópico del producto del raspado o de un pequeño colgajo de piel permite sin dificultad llegar a un diagnóstico etiológico seguro que se podrá corroborar con el cultivo en agar glucosado de Sabouraud.

Formulado el diagnóstico de Candidomicosis de la piel se impone el tratamiento adecuado, según sus diversas localizaciones. En las onixis y perionixis hemos obtenido buenos resultados con los tópicos diarios de:

Acido salicílico	1 gr.
Formol	0,40 c.c.
Alcohol 96°	20 c.c.

Es de utilidad recomendar a las pacientes la necesidad de no mojar sus manos enfermas y protejerlas con guantes de goma cuando los quehaceres de la casa así lo exijan.

En los intértrigos se podrán emplear los tópicos con soluciones alcohólicas de Tintura de yodo al $^1/_3$; con violeta de genciana al 4 %, o pomadas a base de resorcina y crisarrobina al 2 %, sobre todo, cuando se trata de intrértrigos inguinales.

Cepas de orígen varios:

151	— Cepa Barberis. Absceso cerebral. Aislada conjuntamente con *Actinomyces*	*Candida albicans*
171	— Cepa Copello. Lesión ulcerosa de córnea	*Candida albicans*
381	— Aislada de testículo de cobayo inoculado	*Candida flaveri*

Consideraciones sobre localizaciones varias:

El hallazgo de *Candida albicans* en abscesos de cerebro puede explicarse de acuerdo a dos posibilidades: siembra metastásica por vía sanguínea o lo que resultaría más probable, infección ascendente por propagación a partir de localizaciones en vías aereas superiores.

Por último hemos aislado a *Candida albicans* de localizaciones en córnea produciendo una queratítis ulcerosa, localización observada a su vez por otros autores (4), (15).

Conclusiones:

Se estudian y clasifican específicamente 156 cepas de hongos levaduriformes pertenecientes al género *Candida*, aisladas en su mayor parte, de diversas lesiones y productos del organismo humano.

Se comprueba el gran parasitismo por *Candida albicans* en un alto porcentaje y el rol que como agente etiológico tiene ésta especie en ciertas vulvovaginítis; en los intértrigos húmedos blastomicéticos; en las onixis y perionixis; etc.

Se designa con el nombre de Candidomicosis a los procesos patológicos producidos por hongos del género *Candida* y se hacen algunas consideraciones sobre las mismas.

Summery:

The authors have made an study and an specific classification of 156 yeast-like forms of fungus beloning to the genus *Candida* isolated in their majority from differents products of the human body.

They verify also the high percentage of parasitism with is found the *Candida albicans* and the roll this specimen has as an aetiological agent in certain vulvo-vaginitis, bronchomycosis, onychomycosis and perionychomycosis, moist mycosis of interdigital spaces, inguinocrural, perianal, axillary and others cutaneous folds.

With the name of Candidomycosis are designed the pathological process produced by fungus beloning to the Genus *Candida.*

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Nutrient requirements of yeast[1].

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Systematic investigations of the growth requirements of yeast, which were stimulated at the turn of the century by WILDIER's (1901) discovery that yeasts require "bios" for their proper nutrition, have not been carried out in recent years [see TANNER (1925) for critical review of this work]. Recent studies with synthetic media have been limited largely to the evaluation of the ability of the yeasts to produce particular cell constituents, to the determination of vitamin or growth factor requirements, and only recently to classification. Although the general growth requirements of the economically important yeasts, *Saccharomyces cerevisiae* and more recently *Torulopsis utilis*, have been established, a chemically defined medium on which yields comparable to those obtained on natural media was developed only recently. OLSON and JOHNSON (1949) developed a synthetic medium which gave yields of a strain of *Saccharomyces cerevisiae* (isolated from commercial bakers' yeast) which were not improved by addition of natural materials such as corn steep liquor or an extract of malt sprouts.

The nutritional factors essential for yeast growth have been extensively studied. Yeast has long been used as a favorite test object in the fields of general and comparative biochemistry and physiology, and much of our knowledge of carbohydrate, lipid and vitamin synthesis and utilization has been obtained in investigations with yeast. The ready availability of various strains of bakers' and brewers' yeast, their rapid growth under conditions which permit fairly accurate control, and the fact that in their cellular physiology they resemble higher organisms has focused attention upon one species of yeast, *Saccharomyces cerevisiae.* This has been the test object of more biochemical and physiological experiments than nearly any other organism. Even this yeast has been used more often to develop general aspects of physiology and biochemistry than studied systematically. Only twice since its inception in 1932 have the editors of *Annual Review of Biochemistry* allocated space to a review of the biochemistry of yeast, once to FULMER (1939), and once to NEUBERG (1946). The biochemistry of bacteria and related microorganisms has been reviewed annually for the past fifteen years, and five times was space allocated to reviews of the biochemistry of fungi.

Only rarely have other species of yeast been investigated physiologically, and then usually because of some utilitarian objectives; high fat formation (*Endomyces vernalis, Oospora wallroth,* etc.); high protein synthesis from cheap, readily available carbohydrates (*Torulopsis utilis*); high riboflavin synthesis (*Ashbya gossypii*), etc. Only recently have yeasts other than *S.*

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[1] Review of the present state of knowledge in this field presented at the Yeasts in Feeding Symposium (sponsored by the Brewers Yeast Council, Associates of Food and Container Institute of the Armed Forces, Yeast Industry and Q. M. Food and Container Institute for the Armed Forces) held in Milwaukee, Wisconsin, Nov. 8 to 10, 1948.

cerevisiae been used in developing the intermediary mechanisms of metabolism difficult to observe with the latter; carbohydrate assimilation and utilization by *Candida albicans*, VAN NIEL and COHEN (1942); *Brettanomyces* sp., KLUYVER and CUSTERS (1940), and *Zygosaccharomyces acidifaciens*, NICKERSON and CARROLL (1943). In the systematic taxonomic investigations many yeasts have been observed with unusual properties which could be used in basic investigations. A systematic investigation of the biochemistry of yeasts in general is needed for the purpose of discovering natural relationships extending our knowledge of the intermediary metabolism of the more important cellular constituents, and developing more industrially useful yeast products.

Interrelationships Between Nutrilites.

The assimilation of nitrogenous compounds in a chemically defined medium containing glucose as a carbon source, salts, trace elements, and vitamins, was investigated by WICKERHAM (1946). He showed that by the addition of an adequate supply of pure vitamins to the test medium, some yeasts would assimilate nitrogenous compounds that they were previously reported incapable of assimilating. The relation of adequate nutrilite content to nitrogen assimilation is well recognized. SCHULTZ *et al.* (1940) reported that the growth of eight cultures of *S. cerevisiae* in the presence of ammonia or carbamide was dependent upon the type of bios supplement. WINZLER *et al.* (1944) found that the response to biotin of biotin-deficient yeast was dependent upon nitrogen source; ammonia was found to have a specific effect in stimulating fermentation, respiration and growth.

Similar interrelationships between nutrilites and carbon assimilation are known. WICKERHAM and BURTON (1948) reported that the addition of pure vitamins to a synthetic medium would permit the growth of many species that otherwise would fail to grow in the presence of assimilable carbon source. The yeast extract ¹) or other vitamin carriers that have been used previously by some investigators to supply the bios requirements of yeasts would support growth in absence of any test substance.

Although the interrelations between the required nutrilites and carbon or nitrogen assimilation are recognized, similar relations between the minerals, particularly the trace elements or micro-nutrients, and growth and metabolism of yeast, have not been adequately investigated. Only recently has the effect of vitamins on mineral metabolism been explored. NICKERSON and MULLINS (1948) found it possible to increase the radioactive phosphate exchanged by yeast in the presence of glucose by the addition of trace amounts of riboflavin to the suspending medium. The increases observed ranged from 20 to 200 per cent over the phosphate exchanged by controls in glucose alone. This effect was found in two widely different species of yeasts, *Candida albicans* and *Saccharomyces cerevisiae*. They interpreted these results as suggesting the existence of a "P-complexing factor" at the cell surface. The importance in metabolism of processes occurring at the cell surfaces or just inside cell boundaries has only recently been recognized. NICKERSON (1943) has called attention to the importance of a thorough study of yeast permea-

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¹) BOUTHILET, *et al.* (1949) reported that the ability of yeast to grow and ferment yeast extract (an infusion of baker's yeast) in the absence of added carbohydrates was due to the presence of variable amounts of trehalose which could be fermented by several species of yeast. For this reason they have suggested that dilute yeast autolyzate (which does not contain trehalose) be used as the basal fermentation medium.

bility, and BROOKS (1947) has summarized the available information on the relation of permeability to enzyme reactions on cells.

On the basis of our present knowledge of carbon assimilation, nitrogen assimilation, mineral metabolism, and growth factor requirements, an adequate synthetic medium can be defined only in general terms. The optimum pH, temperature, oxygen or Eh requirements are known for but a few species. The temperature and pH requirements, particularly, are based largely on older investigations in poorly defined media.

Carbon Requirements.

The carbon requirements can be satisfied by glucose or sucrose for most yeasts; the utilization of other carbohydrates is still in a rather confusing state, particularly with regard to the disaccharides. The carbon sources used in assimilation tests in the major attempts at yeast classification have been limited to glucose, fructose, mannose, galactose, maltose, melibiose, sucrose, lactose, raffinose, and ethyl alcohol. KLUYVER (1931), on the basis of the comprehensive work of Stelling-Dekker on sporogenous yeasts, concluded that if a yeast does not ferment glucose it can never ferment sugar; that if a yeast will ferment glucose it will also ferment fructose and mannose; and that a yeast will never be able to ferment both maltose and lactose.

While exception to these rules of carbohydrate utilization has not been found with sporogenous yeasts, asporogenous yeasts able to ferment both maltose and lactose are known. The validity of the first rule is sufficiently strong so that WICKERHAM and BURTON (1948) have found it desirable to condition yeasts to grown in synthetic medium with glucose before subjecting them to similar synthetic medium containing carbon sources that may be difficult for the yeast to attack.

Among the hexoses, differences exist in their ability to support growth even in the biologically similar glucose, fructose and mannose. While most yeasts will ferment glucose more rapidly than fructose, the so-called "Sauternes" yeast will ferment fructose more rapidly. Some species of yeast will ferment galactose after previous adaptation; other species do not utilize it. Adaptation to galactose has been the subject of much investigation culminating in the excellent biochemical investigations of Spiegelman and his collaborators.

The pentoses, arabinose and xylose, are usually not utilized by most yeasts, although they are readily assimilated by many molds and certain bacteria. ABBOTT (1926) reported that several species of *Saccharomyces* were able to ferment arabinose slowly at pH 5.0—6.6, and some fermented xylose at these pH ranges. The food yeasts, *Torulopsis utilis* and *Candia tropicalis*, however, do utilize pentoses and can be grown on wood hydrolyzates. *Torulopsis utilis* is known to use pentoses and organic acids as well as hexoses.

The earlier investigations on the utilization of disaccharides have been reviewed by LEIBOWITZ and HESTRIN (1945) and the more recent advances by HESTRIN (1948). HESTRIN (1948) has discussed in some detail the possible mechanisms for the fermentation of maltose, lactose, melibiose and trehalose. Genetic as well as enzymic approaches to the solution of this problem have been made, but additional investigations are necessary before this problem is solved.

Other carbon compounds besides the sugars are known to be utilized. WICKERHAM and BURTON (1948) have investigated a wide variety of carbon compounds, particularly from the point of view of their utility in classification, and have segregated them into nine groups. OLSON and JOHNSON (1949) used a medium containing 10 g. of sucrose and 2.5 g. of l-asparagine per liter as carbon sources.

Nitrogen Requirement.

The nitrogen nutrition of yeast has been investigated from a variety of points of view by various investigators. NIELSEN and his collaborators (1933—1941) at Carlsberg Laboratory have investigated the availability to yeast of the nitrogen in a large number of amino acids under conditions of minimal nitrogen content in a mineral salts-sugar medium containing a small amount of wort as growth supplement. MITCHELL and WILLIAMS (1940) investigated the importance of amino acids as yeast nutrients in a basal medium containing a supplement of pure nutrient factors. THORNE (1945) also used a chemically defined synthetic medium containing three bios constituents in investigations of nitrogen nutrition. WICKERHAM (1946) investigated the assimilation of nitrogen from ammonium sulfate, potassium nitrate, urea, asparagine and peptone, in a chemically defined medium supplemented with eight vitamins. He investigated particularly species of yeast previously reported to be incapable of utilizing ammonium sulfate, urea and asparagine.

Ehrlich, as a result of his investigations in 1907 and 1909 on the production of fusel oil during yeast fermentation, suggested that the sole function of amino-acids in yeast nutrition was to provide a source of ammonia. This was confirmed more recently by THORNE (1937) who extended Ehrlich's investigations to other amino acids. THORNE (1939) also showed that some of the amino acid degradation products, particularly the alcohol tryptophol, are inhibitors of growth and fermentation. The production of inhibitory products, however, cannot be correlated with growth in all cases, and depends on the ease with which amino acids can be deaminated by yeasts. In addition to hydrolytic deamination and decarboxylation, yeasts were shown by THORNE (1945) to bring about a Strickland type inter-molecular oxidation and reduction between pairs of amino acids.

The nitrogen requirements of yeast in general can be supplied by ammonium salts, urea or organic nitrogen compounds. THORNE (1945) found asparagine, aspartic acid, glutamic acid, ammonium phosphate, leucine and arginine, to be good sources of nitrogen for *Saccharomyces* species; ornithine, serine, valine, proline, phenyl alanine, tyrosine, alanine and methionine to be average; tryptophane, histidine, glycine and lycine were found to be bad sources. For the top fermentation brewers yeast, ammonium phosphate was superior as a nitrogen source for both fermentation and growth. In mixtures of leucine and alanine, highest growth and fermentation were found in a mixture containing 80 per cent leucine and 20 per cent alanine. The addition of but 20 per cent ammonium phosphate to glycine which, alone, is virtually useless as a yeast nutrient, gave a mixture practically as good as pure ammonium phosphate. A mixture of 60 per cent alanine and 40 per cent glycine gave practically twice as much growth as alanine alone. NIELSEN and HARTELIUS (1937) reported similar stimulating effect of mixtures of amino acids. A number of amino acids which, individually, were found to have toxic effects on growth, when used collectively were found to have a beneficial effect, increasing the yeast crop by as much as 84 per cent.

Among the organic nitrogenous compounds which were found to have stimulating effects on growth of yeast in a synthetic medium may be mentioned asparagine, aspartic acid and leucine. MITCHELL and WILLIAMS (1940) confirmed these early results by finding that for six strains of *Saccharomyces cerevisiae* grown in a synthetic medium supplemented by ten nutrilites, the five most important amino acids were glutamic acid, aspartic acid, asparagine, arginine and leucine.

Olson and Johnson (1949) found that of twenty-one amino acids tested, only alanine and serine could completely replace asparagine in their synthetic medium for *Saccharomyces cerevisiae* strain Y-30.

Schulz and Pomper (1948) tested a number of different genera of yeast to determine their ability to utilize the nitrogen of individual amino acids as the only sources of nitrogen. Appreciable differences were found in the amino nitrogen utilization of forty species, strains and varieties of *Saccharomyces*, and considerable differences were found between several species of *Torula*, *Candida*, *Hansenula*, *Pichia*, *Zygosaccharomyces* and *Klöckera*. The growth rate of *Saccharomyces* species was slower, generally, on amino acids than on casein and ammonium sulfate. The best yields of *Saccharomyces* yeasts were obtained with alanine, arginine, asparagine, aspartic acid, glutamic acid, leucine, urea and valine; these yeasts, however, were unable to utilize the nitrogen from cystine, glycine, histidine, lysine and threonine.

Barton-Wright (1949) investigated the nitrogenous constituents of wort and their fate during fermentation by top and bottom fermentation yeasts. The principal source of nitrogen for yeast growth in wort was found to be amino acids. In top fermentation 59 per cent of the lysine and 33 per cent of the methionine are removed from the wort in nine hours; in twenty-four hours the whole of the methionine disappears and 87 per cent of the lysine, aspartic acid and leucine. Asparagine nitrogen, although reported to be the best single source of nitrogen for the yeasts, apparently plays a minor role as a source of nitrogen for yeast growth in wort. During top fermentation, in general, yeast utilizes the straight chain amino acids and then attacks the ring compounds. Lysine is almost completely absorbed, and proline is not absorbed in bottom fermentation similarly to their behavior in top fermentation; but glutamic acid is utilized more completely in bottom fermentation. With the exception of aspartic acid, leucine, lysine, methionine, phenyl alanine and tyrosine which are utilized up to 90 per cent, the remaining amino acids are not absorbed by bottom yeast to the same degree as top yeast.

Taylor (1949) investigated the distribution of amino acids inside the yeast cell as well as in the medium by the use of the respective amino acid decarboxylases. Arginine, glutamic acid, histidine, lysine, ornithine and tyrosine, were found to be free inside yeast cells, even when growth takes place in absence of amino acids in the medium. Their concentration in the cell, however, may be increased when the medium is rich in amino acids. The uptake of glutamic acid was found to be dependent on a source of energy (glucose) and the presence of ammonium ions or certain amino acids. The presence of ammonium salts, particularly, decreased both the rate of uptake of glutamic acid and the amount which could be absorbed.

Growth Factors.

The early investigations on growth factor requirements have been reviewed by Tanner (1925), and the more recent investigations have been summarized by Williams (1941). Inositol, thiamine, biotin, pantothenic acid, and pyridoxine have been shown to be required by many yeasts. Other compounds such as nicotinic acid and various pyrimidine and purine compounds have been shown to be stimulatory to some yeasts. The relative concentrations and kinds of nutrilites to be added, however, is still in a contradictory state. The kind and concentration of nutrilites used in recent investigation is given in Table 1. Williams *et al.* (1940) called attention to the fact that yeasts during a long incubation period show considerable ability to grow in the absence of various nutrilites and under such conditions one nutrilite alone may serve, whereas for rapid growth several may be required. This phenomenon has been corroborated and extended recently by LINDEGREN and RAUT (1947).

TABLE 1. *Nutrilite Supplements to Synthetic Media, Micrograms per Liter of Media*

Nutrilite	Wickerham (1946)	Thorne (1945)	Williams et al. (1940)	Mitchell & Williams (1940)	Leonian & Lilly (1942)	Burkholder et al. (1944)	Schultz & Atkin (1947)	Olson & Johnson (1949)
Acetylcholine .				500				
Biotin.	2	1 ml 1)	0.1	1.25	0.5	2.0	5	20
Calcium pantothenate.	400	2,000 2)	6	125	1,000	200	10,000	500
Choline				750				
Ethanolamine .				2,500				
Inositol	2,000	20,000	5,000		5,000	1,000	25,000	10,000
Niacin.	400			2,500			500	—
Para amino benzoic acid .	200							
Pyridoxine hydrochloride	400		40	500	1,000	200	500	1,000
Riboflavin . . .	200			500				
Thiamine hydrochloride	400		40	250	50	200	500	4,000
Uracil				500				
Nicotinic acid						200		

1) Biotin concentrate.
2) as Sodium pantothenate.

In addition to inositol, thiamine, biotin, pantothenic acid, and pyridoxine, other growth factors were suspected by WILLIAMS *et al.* (1941) on the basis of the finding that liver and yeast extracts promote more rapid growth when added to a medium containing all five nutrilites. A higher concentration of the known nutrilites was not found to produce an equivalent stimulating effect. OLSON and JOHNSON (1949), however, found that for their strain of bakers' yeast, biotin, calcium pantothenate, inositol, pyridoxine and thiamine supplied all the growth factor requirements. No additional factor in corn steep liquor or malt sprouts was found necessary to obtain yields of their yeast under conditions of optimum aeration.

Concentrations of Carbon and Nitrogen Source.

The carbon source is usually added in concentrations equivalent to about 25 grams per liter as dextrose. WILLIAMS and SAUNDERS (1934) used 20 g. of sucrose; HARTELIUS (1934) used 100 g. of glucose; LEONIAN and LILLY (1942) used 25 g. of glucose; BURKHOLDER *et al.* (1944) 20 g. of glucose;

THORNE (1945) 60 g. of sucrose; SCHULTZ and ATKIN (1947) 50 g. of glucose; OLSON and JOHNSON 10 g. of glucose. The optimum concentration of carbon source and effect of addition during growth is not well known, particularly for nonaerated media, although the gradual addition of sugar during growth is well established industrially.

The nitrogen source is usually ammonium sulfate added in concentrations of 1 to 2 g. per liter. WILLIAMS and SAUNDERS (1934) used a combination nitrogen source, 1.5 g. of asparagine and 3.0 g. of ammonium sulfate per liter; HARTELIUS (1934) used 5 g. of ammonium sulfate; LEONIAN and LILLY (1942) used 1.50 g. of l-aspartic acid and 0.75 g. of ammonium sulfate; BURKHOLDER *et al.* (1944) used 2.0 g. of asparagine and 2.0 g. of ammonium sulfate; THORNE (1946) added his nitrogen source at the rate of 0.3 g. per liter; WICKERHAM (1946) used 1.0 g. of ammonium sulfate; SCHULTZ and ATKIN (1947) used 3.5 g. of ammonium sulfate; and OLSON and Johnson (1949) used 6 g. of ammonium phosphate and 2.5 g. of l-asparagine per liter.

There is no experimental justification for this range in concentration of nitrogen and carbon sources. Very few quantitative investigations of the optimum requirements for carbon and nitrogen have been made. These, for the most part, were limited to *Saccharomyces* species grown in a medium incomplete in nutrilites and even deficient in micro-nutrient elements whose importance in the metabolism of yeast was then unknown. As pointed out in the review of JOSLYN (1940), FULMER *et al.* (1921) made one of the first quantitative investigations of the mineral requirements of yeast and found that a definite optimum existed for ammonium chloride at 0.188 per cent. Variations from this concentration of ammonia (equivalent to 0.06 per cent NH_3) were found to decrease materially the yield of a strain of *S. cerevisiae.* Similar investigations under controlled pH and in presence of adequate nutrilites are needed to fix the optimum requirements for the major constituents of the medium.

Systematic investigations of the optimum pH for growth have not been made. Usually, and particularly in recent years, the pH of the culture medium is buffered to 5.0. More information on this point is needed.

Mineral Requirements.

The mineral requirements of yeast are not well established. The earlier investigations, which have been reviewed by JOSLYN (1940), are inadequate. Copper, iron, magnesium, potassium, phosphorous and sulfur have been shown to be required. The requirement for other minerals, particularly the micro-nutrient elements, has not been conclusively established. Knowledge concerning the mineral requirement of yeast has been based on the composition of yeast ash, on investigations of bios requirements in which nutrient solutions containing mineral salts and sugar were used in growth studies, on investigations of toxic and antagonistic effects of salts, and more recently on the metabolism of the more important cellular constituents. Aspects of the phosphorous, sulfur, iron, copper, potassium, calcium and magnesium metabolism, have been investigated. The metabolic studies for the most part have been limited to *S. cerevisiae,* but have been useful in formulation of media. The earlier investigations of mineral metabolism have been reviewed by JOSLYN (1940), and the more recent ones by NEUBERG (1946).

The requirement for inorganic phosphate and for potassium is well recognized, but there are still differences of opinion as to the concentration of potassium phosphate which will meet these requirements, as shown in Table 2. The chemistry of phosphate metabolism has been well established for *S. cerevisae.* The conditions for phosphate uptake and the transformations which it undergoes in the metabolism of carbohydrates are fairly well understood. The role of phosphate in fat metabolism and in protein metabolism has yet to be established, and the possible role of phosphates in regulating permeability, moisture relations, and pH within the cell, are unknown.

Table 2. *Mineral Salts Composition of Media for Growth of Yeasts*
(Grams per liter)

Salt	Wickerham 1946)	Wickerham & Burton (1948)	Burkholder et al. (1944)	Thorne (1945)
KH_2PO_4	0.875	1.0	1.5	2.0
K_2HPO_4	0.125	—	—	—
$MgSO_4 . 7H_2O$ . .	0.50	0.50	0.50	1.0
NaCl	0.10	0.10	—	—
$CaCl_22H_2O$	0.10	0.10	0.33	—

	Williams & Saunders (1934)	Leonian & Lilly (1942)	Hartelius (1934)	Schultz & Atkin (1947)	Olson & Johnson (1949)
KH_2PO_4	2.0	1.0	1.0	0.55	0.20
K_2HPO_4	—	—	—	0.50 1)	
$MgSO_4 . 7H_2O$ . .	0.25	0.50	0.70	0.13	0.25
$CaCl_2 . 2H_2O$ . . .	0.25	—	0.40	0.13	
Na_2CO_3	—	0.60	—		
NaCl	—	—	0.50	0.45	
Sosium citrate . .				—	1.00
Potassium citrate.				5.00	—
$(NH_4) . H_2PO_4$ . .				—	6.00

1) KCl.

The use of tracer techniques has well established that the transfer of phosphate into the yeast cell depends almost entirely on carbohydrate metabolism, Lawrence *et al.* (1941), and Mullins (1942). Fermentative activity, rather than respiratory activity, conditions phosphate uptake. It was early observed that potassium ion is indispensable for some partial process of fermentation, and recent investigations have confirmed this (Muntz 1947). Potassium uptake is now known to depend on carbohydrate metabolism. In the initial phases of growth and fermentation the potassium taken up by the yeast is proportional to the quantity of sugar added. Experiments with radioactive potassium have indicated that a rapid interchange of potassium between culture media and yeast cells takes place in two hours, if there is active fermentation. It is now believed that potassium specifically stimulates the reaction of phosphopyruvic acid with adenylphosphates, but this is still in a contradictory state.

Information on calcium and magnesium metabolism is still scanty and contradictory. Magnesium is known to function in carbohydrate metabolism as an activator of at least one transphosphorylase, but the function of calcium is unknown. This is evident also in the wide variation in magnesium sulfate and calcium chloride content of media used for growth studies. The requirements for sulfur, iron, and copper are based upon strong physiological grounds. The requirements for trace elements, however, are still largely unknown.

Boron, copper, iodine, iron, zinc, manganese, molybdenum and thallium, have at various times been shown to promote growth. The role of iron and copper was first extensively studied by ELVEHJEM in 1931, who found that bakers' yeast grew only meagerly on a medium low in iron and copper, and was low in cytochrome content. The addition of 1 mg. of iron per liter of medium stimulated growth and increased cytochrome content to normal levels. The addition of both iron and copper further increased growth in a modified Wildier's medium and increased the concentration of cytochrome a. The optimum amount of copper was found to be 0.1 mg. per liter. Kiene, in 1941, did not observe any effect on rate of growth of yeast with copper, manganese, and molybdenum, but did find iron and zinc to be indispensable. Since neither the carbon and nitrogen sources, nor the salts or vitamins commonly used in preparing media are purified to remove traces of minerals, the usual custom of adding all nine trace elements is based on precautionary measures.

OLSON and JOHNSON (1949) were the only investigators who attempted to purify the basal media from each element to be tested. They reduced the iron content of the basal medium to 0.6 micrograms of iron by serial extraction with a chloroform solution of 8-hydroxyquinoline before testing for response to added iron; the zinc content was reduced to 1.5 micrograms per liter by extraction with a chloroform solution of dithizone at pH 5; and the copper content was reduced to 0.8 micrograms per liter by similar extraction with dithizone at pH 1.5. The concentration of iron required for maximum yield was found to vary with yeast from 25 to 150 micrograms per liter; it was lowest for *Mycotorula lipolytica* (25) and highest for a strain of *S. cerevisiae* —49 (150). The optimum concentration of zinc also varied with strain and species of yeast from 25 to 200 micrograms per liter, being lowest for *T. utilis* (25) and highest for *S. cerevisiae* —49 (200). The optimum concentration of copper was 3 for *T. utilis* and 12—15 micrograms per liter for *S. cerevisiae* Y—30. In the four yeasts tested, the amounts of trace elements required varied within the same species as well as with species. Thallium was not found by OLSON and JOHNSON (1949) to have an effect on growth when added to a medium that had previously been treated to remove thallium ions. Calcium was not found to affect growth even when added to a purified medium; neither did manganese added to a medium containing less than 0.5 micrograms of manganese per liter. Boron, cobalt, iodine and tin added to unpurified basal medium did not affect growth.

OLSON and JOHNSON (1949), however, did find that at least one element, in addition to copper, iron and zinc, was required for maximum yeast growth. The addition of the ash from 1 liter of purified medium, which contained 0.6 microgram of iron, when added to the purified medium gave greater yeast growth than could be accounted for on the basis of its iron content. The purified medium, also, in the presence of excess iron did not give yields equal to those obtained on the unpurified medium.

The current variations in trace element supplements to synthetic media are shown in Table 3.

TABLE 3. *Trace Element Supplements to Synthetic Media* (p.p.m.)

Trace Elements	Wickerham (1946)	Wickerham & Burton (1948)	Burkholder et al. (1944)	Leonian & Lilly (1942)	Williams & Saunders (1934)	Schultz & Atkin (1947)	Olson & Johnson (1949)
Boron	0.01	0.01	0.1	0.18	0.17	—	—
Copper	0.01	0.01	0.01	0.025	0.04	—	0.025
Iodine	0.10	0.10	0.08	0.08	0.08	—	—
Iron	0.05	0.05	0.05	0.20	0.18	—	0.15
Manganese . .	—	0.10	0.01	0.43	0.4	—	—
Molybdenum .	—	0.01	0.01	—	—	—	—
Thallium . . .	—	—	—	0.6	0.6	—	—
Zinc	0.07	0.07	0.07	0.23	0.4	—	0.40

Role of Oxygen in Growth and Metabolism.

Although aeration has been established as a growth requirement, little is known concerning the biochemical mechanism of aeration. The general belief is that oxygen is required for continuous growth of yeast and that the yeast can utilize only the oxygen dissolved in the liquid. Aeration is necessary to replace the dissolved oxygen, to remove fermentation products from the surface of the cells, and to substitute fresh nutrients. Mechanical stimulation of budding may be involved. There are no essentially anaerobic organisms among species of yeast, but yeast may multiply to a limited extent in the absence of oxygen. WINDISCH (1933) showed that yeast cells may multiply for several generations in the absence of oxygen, but noticeable difference in size and form of the yeast cell were observed under anaerobic cultivation.

MEISSEL (1938) has called attention to important cytological differences observable in the same species when developing anaerobically and aerobically. MEISSEL (1938) reported that under aerobic conditions the chondriosomes are distributed more or less regularly throughout the cytoplasm, while under conditions of fermentation they undergo fusion, hypertrophy, and migrate toward the surface. The chondriosomes of respiring cells absorb Janus green intraviral stain and reduce it, but those fermenting cells stain more completely and intensely. Marked changes in synthetic ability also occur with change from aerobic to anaerobic culture. The much higher thiamine content of yeast grown under anaerobic conditions and its lower sterol and fat content are typical of this effect.

Although aeration stimulates growth of yeast, the oxygen content of the gas used affects growth. SLATOR (1921) recognized that yeast in the early stages of growth did not require air. When he investigated growth of yeast in contact with various gases, he found definite growth inhibition in a wordt

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[1]) Nora Neilson and M. A. Joslyn (in press) have found recently that continuous growth of a strain of Jerez flor yeast occurred under nitrogen in the synthetic medium of Olson and Johnson (1949), and other species also could grow well at reduced oxygen tension. Marked differences in cytochromes present between cultures grown in air and in nitrogen occurred.

medium as the oxygen content was increased. The generation time in the absence of air was 2.7 hours; in the presence of air 2.8 hours; in presence of 60 per cent oxygen and 40 per cent nitrogen, 3.1 hours; and in pure oxygen, 3.5 hours. SLATOR (1921) believed that air had very little direct influence upon crop yields or rates of growth. He reported that it played no part in the initial budding of the yeast, in the lag phase of growth, and did not cause any direct acceleration in the first stages. In later stages of growth its stimulating effect was due largely to removal of carbon dioxide which depresses growth.

The fact that pure oxygen is not as good a growth stimulant as air has been confirmed industrially, de BECZE and LIEBMAN (1944). The growth of yeast under anaerobic conditions is characterized by being limited, and the limiting factor may be the concentration of some growth factor synthesized aerobically and distributed among the anaerobically produced descendents in amounts decreasing with each generation until growth ceases. The excellent survey of methods of aeration in the production of compressed yeast by de BECZE and LIEBMAN (1944) indicates that despite the industrial development many unsolved problems remain. The quantity of air used, and the conditions under which it is introduced, distributed, and kept in the medium, are important to yield. The most efficient use of air is obtained when the air particles are small and remain in the liquid for the longest period of time. Because of colloidal and other effects, the yield does not increase directly with decrease in size of air bubbles.

Agitation as well as aeration influences growth rate and yield, but has received much less consideration. Both agitation and aeration have been investigated by SINGH *et al.* (1948). These investigators found a species difference among the yeasts investigated. Nearly maximum yield of *S. cerevisiae* was obtained with aeration alone, and of *T. utilis* with agitation alone, while both aeration and agitation were required for maximum yield of *C. arborea.* Further investigation of this interesting specificity is desirable.

A very striking feature of the yeasts is the range of respiratory and fermentative intensity which they exhibit. Species of yeast with a very high power of oxygen absorption, a high oxidation-reduction potential, a high cytochrome and aerobic oxidase content are known as well as those with a very high fermentative power a low O—R potential, a low or negligible cytochrome content, and a high glutathione content. It is well established that oxygen influences not only the nature of the products but also the rate of fermentation and substrate decomposition.

OLSON and JOHNSON (1949) differentiated between the rate at which air is supplied to a fermenter (air flow) and the rate at which oxygen dissolves in the fermenter liquid (effective aeration) as measured by the sulfite oxidation method. The effective aeration of the medium was found to vary with the volume of liquid in the flask, the rate of flow of air through the flask and the type of shaking (agitator or shaker speed). At a given air flow rate, the liquid volume determined the rate of effective aeration which increased to a definite maximum as liquid volume increased and then decreased. The yield of yeast obtained at different levels of effective aeration, however, increased to a maximum at 1 millimol of oxygen per liter per minute and then decreased at first gradually and then sharply. The yield curve vs effective aeration in a 500 ml Erlenmeyer flask shaken on a Gump rotary shaker followed closely that in the 3.5 liter fermenter equipped with agitator. The decrease in yield at higher levels of aeration was shown not to be due to loss of alcohol.

The maintenance of a desirable oxidation-reduction potential is another possibility in explaining the effects of aeration. Although the oxidation-reduction potentials of metabolizing yeast suspensions have been measured, HOOGERHEIDE (1936) in particular, has shown that the O—R potential is characteristic of the type of metabolic activity, systematic investigations of this aspect of nutrition have not been made.

HANSON and BALDWIN (1943) have recently found that the effect of certain metals in yeast is influenced by the redox potential. In a grain wort medium, addition of up to 96 micrograms of iron per ml, 60 of zinc, and 30 of aluminum, had no effect on growth. But when the redox-potential was raised to 600 mv, iron induced a decrease in lag phase, zinc had no effect, and aluminum increased the lag phase. When the potential was lowered to100—150 mv, the addition of iron and zinc shortened the lag phase, but aluminum increased it. These observations are very interesting and should be extended to other phases of mineral metabolism.

Growth and Assimilation.

The economic production of food yeasts of the desired quality necessitates the development of cultural conditions capable of producing high yields of yeast in a readily separable form. In the development of such conditions the experience gained in the manufacture of bakers' yeast was used as a guide. In the production of bakers' yeast, conditions were developed industrially which essentially involved the promotion of growth at the expense of alcohol formation. Aeration, introduced in about 1900, gradually improved the yield of yeast (fresh basis) from 14 per cent of the weight of the added substrate in 1900 to about 50 per cent in 1920.

During the first World War it was discovered independently by German and Danish yeast factories that the yield could be further improved by adding the substrate solutions in small portions to the growing yeast. By successively modifying the scheme of the substrate addition and by making the aeration more intense it has been possible to obtain yields approaching and surpassing 100 per cent of the added substrate, at least for molasses. This steady improvement of cultivation methods in the industry was not accompanied by a similar growth in the science of yeast cultivation. The difficulty of duplicating in the laboratory the aerobic conditions used in the industry, and the fact that the feeding systems were often kept secret, made laboratory progress lag.

The introduction of the yeast, *Torulopsis utilis*, by FINK and his coworkers changed this condition because this yeast could be cultivated in the laboratory with yields as great as that obtained industrially. It is also a poor producer of alcohol so that experimental results could be better interpreted. Most of the older work on growth of *S. cerevisiae* and the more recent work of FINK was directed towards the chemical study of the biological cell substance synthesis with the aim of setting a limit to growth. This was realized when FINK *et al.* (1939) observed in macro growth experiments that 90 per cent of the carbon postulated to be assimilated by washed suspensions according to the equation,

$$C_6H_{12}O_6 = 2CH_3CHO + 2CO_2 + 4H$$

was actually assimilated during the growth of *Torulopsis utilis*.

Under optimum conditions of growth FINK and KREBE (1939) believed that a maximum of 52 per cent of glucose could be converted to yeast cell substance. SPERBER (1945) obtained a conversion of 59.8 per cent. The dry weight yield of *Torulopsis utilis* in terms of sugar used has been reported to vary from 38.1 per cent to 65.3 per cent; the latter yield being obtained by SINGH *et al.* (1947) under optimum conditions of aeration and stirring. The yield of *S. cerevisiae* has varied from 17.4 per cent to 54.3 per cent.

The most fundamental approach to growth was due to KLUYVER's concept of stoichiometric rather than energetic relation between dissimilation and assimilation. It is generally assumed that the oxidation of foodstuffs makes energy available to the cell for synthetic processes, but the energy coupling between the assimilatory and dissimilatory processes is difficult to picture even in terms of the modern concept of high energy phosphate systems. In 1930, KLUYVER suggested that dissimilatory processes may well serve as a source of raw material for the assimilatory processes; that is, that anabolic reactions may occur spontaneously if the proper initial substances are provided by the catabolic activities of the cell.

KLUYVER's pioneer concepts in this field have been well supported by a number of studies in recent years (reviewed by CLIFTON (1946). This concept has been useful in studies of growth and assimilation by *S. cerevisiae* as well as other micro-organisms. These studies in general have shown that definite quantitative relationships exist between the amount of a foodstuff dissimilated and the amount assimilated by non-proliferating cells. With some organisms, synthesis during growth closely approaches assimilation observed in cell suspensions. Both oxidative and fermentative assimilation has been observed with yeast.

WINZLER and BAUMBERGER (1938) and VAN NIEL and ANDERSEN (1941) found that the production of alcohol and CO_2 from sugar by yeast under anaerobic conditions accounts for only about 70 per cent of the carbohydrate which disappears, the other 30 per cent being converted initially into complex, more slowly fermentable, carbohydrates. PICKETT and CLIFTON (1933) reported the oxidative assimilation of approximately one-third of the glucose carbon by washed yeast cells. SWANSON and CLIFTON (1948) in their studies of actively proliferating cultures of *S. cerevisiae,* observed that these, when cultivated aerobically, carry on the most active initial stages of their metabolism by a process of fermentative assimilation. This stage predominates until hexose disappears, and oxidative assimilation then occurs from the intermediate products of saccharide dissimilation. This investigation accounts for the validity of the new Danish process for growing yeast which makes use of an initial anaerobic phase followed by aerobic phase of growth. These investigations and analyses, when extended, may have an intimate bearing on the larger problem of the biochemistry of growth.

Sommaire

L'état présent de la science concernant les exigences nutritives de la levure est passé en revue, accentuant les aspects incomplètement examinés. Bien que la levure a été, depuis longtemps, l'objet favorit de recherche sur le champ de la chimie générale et comparative, les études systématiques sont encore bien incomplètes. Les espèces, autres que *Saccharomyces cerevisiae* et *Torulopsis utilis,* ont été rarement examinées physiologiquement. Les relations entre le contenu de l'aliment et l'assimilation de l'azote (nitrogène) et du carbone sont bien reconnues, mais des pareilles rélations entre les ,,rares éléments'' (trace elements) ou micro-aliments et croissance, ont été examinées incomplètement. L'importance de métabolisme des procèdes qu'on rencontre à la surface de la cellule ou à l'intérieur près de la surface, ont été reconnus récemment. Un milieu synthetique qui a produit un rendement de *Saccharomyces cerevisiae* sans supplément du matériel naturel a été développé récemment. L'exigence de l'azote (nitrogène) et de crabone pour la croissance a été assez bien examiné qualitativement, mais très peu de recherches quan-

titatives concernant l'optimum des exigences de sources du carbone et de l'azote (nitrogène) ont été faites. Les relatives concentrations, et les genres de facteurs de croissance qui devraient être ajoutés sont encore discutés, et on y trouve des grandes différences de concentration de facteurs de croissances qui sont ajoutés au milieu chimiquement bien définis. En général, le minimum des exigences de la levure n'est pas encore établi.

On soulignait particulièrement le rôle de l'oxygène comme facteur de croissance et de métabolisme. Ce problème est étudié à present avec intensité en Angleterre, France et aux Etats-Unis, et spécialement en ce qui concerne l'éffet et l'étendue de l'aréation sur l'accumulation de cytochrome-oxydase pigment dans la levure.

Ce n'est que récemment qu'on a trouvé qu'une bonne continuelle croissance de la levure peut être obtenue en absence d'oxygene. On a des preuves dans l'industrie que la levure. des brasseurs peut être cultivée avec bon rendement dans l'atmosphère de CO_2. L'effet du potentiel oxidation-réduction, de pH, de température et d'autres facteurs physiques, n'a pas été encore suffisamment examiné. Les résentes recherches concernantes l'aérobique et anaérobique assimilation des hydrocarbones par la levure, et leur relation aux exigences de croissance, sont passées en revue.

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Critical Survey of the Mycological Literature of the years 1939 to 1942

by

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E. Bronchomoniliasis

DECKER'S (1939) case report on *Pulmonary Moniliasis* is of interest.

BAKST, HAZARD, and FOLEY and DAVIS and WARREN have reviewed the literature and classified the pulmonary disease into three types: The mild type, which may not affect the general health of the individual to any great extent. There is a cough with mucopurulent sputum, and usually no hemoptysis or fever. Physical examination of the chest may reveal scattered fine rales. The condition may persist for several months or may progress to a more severe form. The intermediate type, which may simulate either bronchitis or early pulmonary tuberculosis. Fever, cough, mucopurulent sputum, occasionally blood-streaked sputum, dyspnea, and localized or generalized fine or coarse rales are frequently observed. The severe type, in which the history and physical signs are very similar to those of well-advanced pulmonary tuberculosis. General malaise, loss of weight, dyspnea, fever and cough are usually present, and often blood-streaked sputum, chest pains, rales and signs of pleural thickening and consolidation. A 57 year old farm worker was seen September 27, 1935, with a chief complaint of weakness. He stated that he was perfectly well until 1 week before entry, when he developed a cold with cough but no sputum. He then became progressively weaker, and developed dyspnea on slight exertion, and anorexia. His usual work became more difficult to accomplish, and just prior to entry he was unable to do any work whatsoever due to marked fatigue. There were no other complaints. The past history was essentially negative, except for a fracture of the 10th left rib 9 months previously, at which time a roentgenogram had been taken. Physical examination revealed a very well-developed, well nourished man, who appeared weak and definitely ill. The nose and throat were normal. The lungs were clear. Tactile and vocal fremitus and breath sounds were within normal limits, and no rales were heard either before or after cough. The heart was normal. The blood pressure was 140/85. Abdominal and rectal examinations were negative. The temperature was 101 F. The white-cell count was 8850, with a normal differential. Urinalysis was entirely normal. Bed rest and treatment for upper respiratory infection were started. The temperature remained elevated, the cough continued without sputum, and the physical examination was unchanged until the 3rd day after admission, when examination of the chest revealed diminished tactile fremitus over the left midchest and base posteriorly, with diminished breath sounds and fine rales in this region. The findings were suggestive of pneumonic process, and a roentgen ray examination revealed obliteration of both diaphragm shadows. The lung markings were increased at both bases, there was haziness in the left lower lobe, and a dense shadow filled the right base, the upper border of which was markedly irregular in outline. There was also a moderately dense, enlarged right hilus shadow. After viewing the roentgenogram, examination of the chest was repeated, but no rales could be heard on the right side. On the following day (the 4th after admission), however, fine rales were heard over the right base as well as the left, and over the left midlung field posteriorly. The patient raised a small amount of thick, brown, tenacious sputum, which was smeared and cultured. No acid-fast bacilli were seen, but in all stains there were many yeast cells. Suspecting that the yeast was a contaminant, the patient was instructed to wash his mouth and throat well with saline solution, after which another sputum specimen was obtained, similar to the first. Yeast cells were found in all the stained smears of this specimen. Because of the similarity of these organisms to moniliae, the patient was given potassium iodide, 15 gr. three times daily. The lung findings remained essentially unchanged, the cough continued and the sputum increased, with a larger number of budding cells and mycelia in each subsequent specimen until the 10th day, when the temperature, which had gradually subsided, had become normal. A roentgen film of the chest on this day showed calcification at the line of fracture of the 10th left rib posteriorly. The cardiac borders were indistinct in outline and merged with moderately dense hilar and peri-hilar shadows. Peribronchial thickening and fine mottling was evident in the right lung, extending toward the base. The lower two thirds of the left lung field was mottled, and there were dense, confluent patches throughout this area. There was a faint outline of a large node in the right hilus, and a dense shadow in the left hilus consistent with a calcified node. The patient began to improve clinically. His appetite increased, the cough and sputum decreased; physical examination showed diminution of abnormal lung findings, and on the 17th day was negative except for fine rales in both bases. Roentgenograms taken this day showed the right border of the heart more distinctly. The hilar shadow on the right was less dense. Both diaphragm shadows were seen, the left being clearer than the right. There was a minimum of haziness in the right costophrenic angle. Peribronchial thickening was unchanged at the right base. More diffuse mottling and peribronchial thickening were seen in the left base, with loss of the larger conglomerate patches. The cough continued to subside and the sputum became increasingly less until the 24th day following admission, when the sputum showed only one colony of yeast cells on culture. Roentgen films at this time showed the right diaphragm more distinctly than theretofore, and a clear costophrenic angle and slightly diminished peribronchial thickening. The mottling in the left lung field was more diffuse, with a considerable amount of beading along the peribronchial vessels. The patient's activity was gradually increased after the 25th day, and by the 31st day he felt perfectly well and had no fatigue following moderate exercise, while examination revealed only an occasional fine rale in either base, and a roentgenogram showed the heart and diaphragm shadows more distinctly. The peribronchial thickening and mottling had diminished, and there were very few residual pathologic findings. The patient was discharged on the 31st day (October 28), and returned to work December 3. Roentgen films of the chest taken then and December 26 showed an increase in pulmonary markings in both bases.

BEATTY (1939) reported on *Chronic Pulmonary Moniliasis.*

The diagnosis of pulmonary moniliasis first rests upon having the condition in mind. Since it may simulate other chest conditions, finding the organism in the sputum is the most valuable criterion for a diagnosis. According to the literature the typical case is that of an individual whose clinical symptoms may be mild in comparison to the X-ray findings. The usual location of the X-ray lesion is in the bases. The sputum is oftener mucoid or serous unless the patient is having an acute attack when it may be purulent. The physical findings may be scant also. Dyspnea on exertion and extreme weakness may be out of proportion to otherwise mild symptoms. It must be stressed that the final diagnosis depends upon finding and identifying the organism in the sputum and even then we may have a contamination from the mouth, secondary invasion of the lungs or bronchi or a primary fungus infection.

Case Report

This patient, a male, forty four years old, came in with the chief complaint of shortness of breath of four years duration and soreness in the neck of three years duration. He stated he was perfectly well five years ago and four years ago noticed shortness of breath on exertion. Three years ago a swelling appeared at the base of the neck on the right side. He repeatedly had hemoptysis. He described his sputum as mucoid and stated this is raised by clearing the throat. He has tired easily for four years. Appetite is bad at times. When the patient has a bad cold he notes a swelling in an enlarged gland at the base of his neck on the right side. Coincident with this for the past three or four years he has a soreness in the clavicle and upper anterior ribs which subsides as the swelling subsides.

His industrial and occupational history reveals that he was born in Southern Kentucky and lived there all his life as a farmer.

A case history of a chronic pulmonary mycotic infection due to monilia albicans is presented. In the differential diagnosis several things have to be considered on account of the patient's history. Since he lived in Missouri where sporothricosis occurs this will have to be considered. He also worked in New Albany sandblasting for a while. A silicosis with secondary moniliasis will have to be considered. Then he gave a history of a bloodstream infection which followed hunting. He had chills and fever, delirium, and it was noted after the onset of this illness he had a sore on his finger with red streaks and glandular involvement. Later an axillary gland was lanced. He could have had at this time an ordinary infection, or a fungus infection of finger with systemic spread, or a tularemia. And, too, all the other pulmonary fungus infections will have to be considered in addition to tuberculosis, but the most likely diagnosis seems to be *monilia albicans.*

BALTZAN (1940) presented a noteworthy case report on *Bronchomoniliasis in Saskatchewan.*

Bronchomoniliasis is a bronchopulmonary disease caused by the fungus of the genus monilia. CASTELLANI's first investigations were instigated by the constant findings of monilia in large amounts and without the concomitant presence of tubercle bacilli in the sputum of persons suffering from a disease entity simulating pulmonary tuberculosis. These persons were employed in tea factories in Ceylon. From Ceylon in 1905 CASTELLANI published the first description of this infection, and since then cases have been recorded in all climates. The farthest north that the disease has been described on this continent is Baltimore, Md. and St. Paul, Minn. No reports of bronchomoniliasis in Canada are on record. The only continent from which reports are lacking is Australia.

Under the guise of tuberculosis, bronchomoniliasis is more common than is generally recognized. A realization of the cosmopolitan nature of monilia infection, indistinguishable as it is clinically from tuberculosis, will lead to a growing appreciation in all parts of the world of the value of prompt recognition of this pathological entity, for in its mild form the disease readily responds to the treatment with iodides, but if neglected or treated as pulmonary tuberculosis it usually ends fatally.

Acute primary pulmonary infections by yeast-like organisms may begin rather suddenly with chilliness, aching, malaise, cough and high fever. Soon dyspnoea, cyanosis, and signs and symptoms of an atypical pneumonia present themselves. Physical signs in the chest are variable and may consist only of coarse râles, atypical pneumonitis, patchy or massive consolidation. The apices of the lungs are seldom involved. In chronic cases cavity formation is not unusual. It is emphasized that in chronic cases the shadows resemble those of chronic pulmonary tuberculosis, with fibrosis, cavitation and pleural involvement. Roentgenograms of the chest usually show the process beginning in the hilar areas, spreading outward along the bronchovascular markings until the whole lobe may be densely obscured. The density may spread in the same lung or begin afresh in the opposite lung. The roentgen pictures are therefore fairly characteristic; primary bronchomoniliasis does not settle in the apices, the roots of the lungs are particularly vulnerable, the dissemination is of a wandering nature, and the densities have a fluffy cotton-like appearance. The clinical diagnosis is easily missed unless the disease is kept in mind and the sputum is examined microscopically and bacteriologically. The sputum should be obtained as fresh as possible after the patient has washed his mouth thoroughly with a weak solution of potassium permanganate or sterilized water. He is then asked to cough or expectorate and the sputum is examined without delay in order to avoid contamination. It must always be remembered, however, that monilia in sputum may have no clinical significance, but in doubtful cases the pathogenicity can be proved by animal inoculation.

DOUB (1940) investigated the *Roetgenologic aspects of bronchomicosis.*

The bronchomycoses, while not rare, are not discovered with great frequency, and until the past few decades comparatively little attention was devoted to them. The symtoms and signs are similar to those of tuberculosis and often they are at first thought to be tuberculous in origin. Whenever the history or x-ray changes are atypical of tuberculosis, the sputum should be examined microscopically and also cultured repeatedly, so that these cases may not be treated as tuberculous cases. In early stages, these patients appear to have a mild bronchitis with some expectoration; later they develop many of the signs and symptoms and even the complications of tuberculosis. The final diagnosis rests on the discovery and identification of the offending organism. The roentgen changes are suggestive of a differential diagnosis in some instances but usually one can only suggest the presence of a fungus infection.

VADALA (1940) presented a comprehensive study on *Mycotic infection of the broncho-pulmonary tract.*

During the past five years there have been 350 cases referred for diagnostic bronchoscopy, which have comprised cases of bronchitis, silicosis, pulmonary tuberculosis, and asthma. At the time of the endoscopic examination, routine cultures were taken from each of the large bronchi and examined for fungi. A survey of all cases reported in the hospital since 1919 reveals that there have been numerous clinical diagnoses of bronchiectasis and pulmonary tuberculosis, without the presence of tubercle bacilli in the sputum; in a certain number of which postmortem examination revealed pulmonary mycosis. Roentgenograms of mycotic or pulmonary tuberculosis suspects which did not have tubercle bacilli in the sputum are available on all these cases. The clinical histories of eleven cases are reported in detail as they illustrate the characteristic changes present in such cases reported in this article. There is a difference in the pathological picture presented by the various mycoses in their invasion of the bronchopulmonary system which will be evident in a detailed analysis of the findings in the several principal types of this group infections.

Actinomycosis — Primary pulmonary actinomycosis may develop about particles of inhaled vegetable matter. One case is on record where an awn of barley was found in the center of a primary pulmonary lesion. In some cases, lesions of the lung are secondary to lesions of the mouth parts. Lesions of the lungs vary in character from a subacute bronchopneumonia to a chronic but invariably fatal disease resembling tuberculosis. In 1920 HENRICI and GARDNER collected records of 26 cases of actinomycosis in man. In all but three of these the infection was primary in the lungs or peribronchial lymph nodes. In the lungs there is produced a caseous bronchopneumonia with eventually softening and cavity formation, but there is a pronounced tendency for the organisms to become distributed through the blood with the formation of abscesses in other viscera, expecially the brain. In thoracic actinomycosis, early recognition of the lesion is likely to be accidental. Often it is the occurrence of an empyema which is threatening to rupture through the thoracic wall, which gives the first suspicion of the presence of pulmonary actinomycosis. The roentgenologist may occasionally diagnose the lesion when the lung, pleura, and chest wall are involved; that is, when there is an intrapulmonary lesion which simultaneously gives rise to a periostitis, actinomycosis should be suspected. Pulmonary abscess, bronchiectasis with abscess or gangrene of the lung, fungus infection of the lung, broncho-pulmonary spirochetosis, and hydatid cyst, may be confusing to the diagnostician. Monilial infection of the broncho-pulmonary system was first described by CASTELLANI in Ceylon in 1905, which occurred in tea sorters. Since then pulmonary moniliasis has been observed, studied, and recorded, by a number of investigators in different parts of the world, especially in the tropical countries. Subsequent studies in Europe and especially in the United States showed that especially in this country bronchomoniliasis of CASTELLANI is much more diffused than has been commonly admitted (STOVALL and GREELEY, STOKES, KISER, and SMITH). The bronchopneumononiliasis of CASTELLANI is now recognized as a nosologic entity. It has been defined and repeatedly confirmed not only in the tropical countries but also in temperate climes. Cases have been reported from France, England, and Greece. Eight or nine cases have been reported in Italy. The clinical symptomalogy is generally identical with that of chronic common bronchitis, with periods of recrudescence, and participation of the pleura and diffusion of the process to the pulmonary parenchyma in some cases. These latter cases are considered as grave in nature, and have frequently been erroneously classified with pulmonary tuberculosis. The pathologic contributions on this subject are few in number and mostly refer to experimental investigation.

Bronchomoniliasis is no longer considered a rare condition, although the diagnosis must be made with extreme reserve, since the organism is frequently demonstrated in the secretions of the upper air passages of a normal individual, and particularly in the sputum of patients suffering from chronic pulmonary disease, notably tuberculosis. Early primary lesion of bronchomoniliasis has not been described, and probably cannot be distinguished from those caused by any other form of inflammation. It is possible that a primary bacterial or allergic inflammation of the bronchi or the pulmonary parenchyma may first prepare the "soil" for the invasion and growth of monilia, which, once firmly platted in the tissue, continues to live a tenacious, semi-saprophytic existence, and to stimulate mildly chronic irritations with a resulting low grade inflammation. This conception is supported by the finding of monilia in the walls of the bronchi and in the regional lymph nodes at necropsy in a case of chronic bronchial asthma of several months duration. Such findings may be considered the pathological basis of bronchomoniliasis in the early stage. In an endeavor to fix a more exact etiology for these illnesses the freshly expectorated sputum of each case was studied as follows: 1. the gross characteristics of the specimen were observed; darkfield examination and gentian violet stains of fresh smears for fuso-spirochetal organisms were made; examination of fresh smears in 10 per cent sodium hydroxide for spores and mycelia is next in order; examination for tubercle bacilli and other organisms by the ZIEHL-NEELSEN method; and GRAM stain. 2. Suspected particles of fresh sputa are next streaked on blood-agar slants and incubated at 38 degrees C.; and for fungus culture they are streaked on Saboraud's medium and incubated at 37 degrees C. and 25 degrees C. Cultures for fungi should be observed for two weeks, as some of the members of this group are slow in growth, and GILL believes many diagnostic oversights are traceable to failure to observe this simple rule.

Coccidioidomycosis — Geographically, the disease has been recognized in South America and Italy, and in about a dozen states of U.S.A. In the San Joaquin Valley, California, coccidioidal granuloma has been found to occur with considerable frequency. Cattle and sheep as well as human beings acquire the disease. There is no indication of transmission of the disease from man to man, animal to man, or vice versa. Persons of all ages and of either sex may be affected. It is known as "Desert Fever" or "Valley Fever." It occurs in highest incidence in regions where there is prolonged hot, dry weather, favorable to the dissemination of the spores. From a study of the roentgenograms of many of these cases, EVANS and BALL make the following comment: "A diffuse shadow, at times simulating pneumonia, occurs in many cases, and involves the right lower lobe twice as frequently as any other part of the lung field, though it may occur in any portion, and twice as frequently in both hilar regions as in one. In many cases the lesions resemble those of a disseminated miliary tuberculosis. The use of the coccidioidin cutaneous test in case of "Valley Fever" has been most helpful in correlating the diagnosis with coccidioides infection. It is a specific skin antigen and extremely active preparation. The reaction is said to be more violent in valley fever than in cases of coccidioidal granuloma. DICKSON found that 0.1cc of a 1 to 1,000 dilution of coccidioidin is adequate and produces well marked reactions, sometimes with vesicle formation in cases of primary infection. At least 0.1 cc of a 1 to 10 dilution of this coccidioidin is necessary for coccidioidal granuloma. DICKSON no longer uses the control since very allergic types of individuals have failed to react to the control. In cases of primary coccidioides infection without erythema nodosum in which fungus coccidiodes is found in the sputum the diagnosis may be made of bronchopneumonia or influenza.

Sporotrichosis. The pulmonary form of sporotrichosis is occasionally seen in this country, though by far the greatest number of reported cases have come from Europe. Fifteen cases have been collected by DE BEURMANN and GOUGEROT.

Aspergillosis — there are three varieties of aspergillus which may, under conditions of lowered pulmonary resistance, cause an infection of the lungs; *A. Fumigatus*, *A. Flavus*, *A. Niger*. This infection appears to have a definite affinity for involvement of the pulmonary system. The local pathology is characterized by the appearance of granulomatous nodules on the mucosa, which runs a chronic course. In this country, aspergillus invasion does not seem always to be of the pathogenic type, and in a suspected case it is always a question of considerable discussion as to whether or not the fungus is purely a contaminant or a secondary invader. In a public health survey of the iron mines in the Pitcher, Oklahoma, district, in which many chest roentgenograms were taken, SAYRE and MERRIWEATHER were puzzled to find in many plates, discrete, dense shot-like areas throughout both lung fields of workers who had never been ill. From the sputum they were able to isolate the *A. Fumigatus*, Fisheri and *A. Niger*. Antigens from both of these fungi were made, and when used, only the *A. Niger* caused a dermal reaction. When these plates were submitted to a group of eminent roentgenologists the diagnosis was as follows: chronic miliary tuberculosis which has healed by calcification; pneumonoconiosis; pneumomycosis. The condition has also been found among the wheat threshers of Oklahoma and Nebraska. OPIE, in commenting on this particular condition, remarks that we are finding a type of inactive miliary tuberculosis, with increasing frequency. It is his opinion that a great many more cases recover than is believed. Occupation has a distinct relationship to the etiology of aspergillosis. It occurs most often in bird fanciers, pigeon breeders, hair sorters, millers, and in people who inhale dust, grain particles, and vegetable matter, The onset of the infection is insidious in character, accompanied by cough asthma, anorexia, and an evening temperature, and in the early stages is frequently treated as a chronic bronchitis. Later in the chronic course of the disease there is a loss of weight, night sweats, and hemoptysis. Certain mycotic infections of the lungs have long been recognized as entities. Actinomycosis and blastomycosis are the common examples. Their known pathogenicity to man, and their ability to produce readily local lesions, and to cause a systemic invasion and visceral involvement, and eventual death, have established them as definite clinicopathologic entities. Mycotic disease affecting the respiratory tract can be definitely diagnosed by bronchoscopic examination and study. Aspiration of exudate in the bronchi, uncontaminated by infections found in the mouth and pharynx, combined with bacteriologic study is the certain method of recognizing these maladies in diseases of the pulmonary system. Most cases of this nature or type are sent into tuberculosis sanatoria with suspected tuberculosis, and may be treated as such for a long period of time, or until the mycosis is finally diagnosed and proper therapy instituted. Positive diagnosis by clinical observation, repeated negative sputum for tubercle bacilli, the bronchoscopic aspiration, and the proper treatment other than that for tuberculosis, is important in mycotic disease, since in the mycoses the iodides offer the most hopeful method of cure, which in tuberculosis should not be used because of its detrimental effect. Proper x-ray interpretation aids immensely in diagnosis. Final diagnosis depends upon aspirating affected exudate through the bronchoscope, and developing cultures to differentiate between tuberculosis and mycosis. Treatment, in mycotic infections, depends on organism isolation and is outlined in detail in the different subdivisions of this article. A bronchoscopic department should be included in the clinical setup of all hospitals treating diseases of the broncho-pulmonary system.

F. Blastomycosis Gilchrist

(*The North-American Type*)

Martin and Smith (1939) presented a comprehensive review of the literature on *American Blastomycosis* (Gilchrist's disease) with report of thirteen new cases.

The present study is based only upon those cases falling into the classification of American blastomycosis, or Gilchrist's disease. Three hundred and forty-seven case reports have been studied, including the 13 patients described in this paper. Only 80 (23 per cent) of these cases can be considered as unquestionably proved to be caused by *Blastomyces dermatitidis*. One hundred and sixty-three cases (47 per cent) were classified as presumptive on the bases of clinical histories, descriptions of the lesions, photographs, biopsy studies, or positive smears. In 104 cases the descriptions were too brief to permit inclusion among the proved or presumptive cases. Cases reported as blastomycosis, which were obviously due to fungi other than Gilchrist's organism, are not included in the statistical study.

Table I. *Classification of Cases reviewed*

	Systemic	Questionably Systemic	Cutaneous	Total
Proved Cases	60	4	16	80
Presumptive Cases	57	13	93	163
Inadequately described cases	20	16	68	104
Total	137	33	177	347

The incidence of proved and presumptive cases in the various age groups revealed that no systemic infections have been described in a patient under the age of twelve years or over the age of seventy. Sixty-eight patients, or more than 50 percent of those with systemic blastomycosis whose ages were reported, were between the ages of twenty and forty years. The age distribution is considerably wider in infections essentially limited to the skin. The youngest patient with proved blastomycosis was reported by Rosenthal (205) in an infant six months of age. Varncy (232) described a case of skin infection in a nineteen year old girl whose initial lesion was said to have followed a minor injury at the age of six months. Males are much more frequently infected with *Blastomyces dermatitidis* than are females, the ratio being approximately 9 to 1 (see table 2). Including only the proved amd presumptive cases, 92 per cent of the patients with systemic and questionably systemic infections and 80 per cent of patients with skin or localized lesions were males. Of the 181 cases in which race was recorded, 50 (28 per cent) were Negroes and 131 (72 per cent) were white. The 3 Mongolian cases reported (24, 222, 231) were unproved. Fifty-four per cent of the white patients were of foreign extraction with Italians and Germans predominating. The disease is far more common among the poorer classes.

TABLE II. *Sex distribution of blastomycosis*

	Systemic		Questionably Systemic		Cutaneous		Total
	Male	Female	Male	Female	Male	Female	
Proved	57	3	4	0	12	3	79
Presumptive .	51	6	10	2	72	18	159
Inadequately described . .	16	4	13	2	51	10	96
Total.	124	13	27	4	135	31	334

GILCHRIST'S disease is an infection essentially limited to North America. Proved and presumptive cases have been found in twenty-eight states, and if one accepts the inadequately described cases this number is brought up to thirty. There is an impression among some clinicians that the disease is rarely seen outside of California and Illinois, but in fact only 20 per cent of the recorded cases have been from Illinois (hence the name "Chicago disease"), and more cases of blastomycosis have been found in nineteen other states than have been reported from California. Further evidence for the wide-spread distribution of the disease is obtained from discussions following papers on this subject. WILLMOT in Kentucky states that he had seen 25 or 30 cases; WENDE observed 25 cases around Buffalo, New York, and CRUTCHFIELD, of Texas, remarked that the disease was very common along the Gulf States. Analysis of the foreign cases is complicated by the failure to distinguish clearly between the American type of the disease caused by the mold-like *Blastomyces dermatitidis* and the European type caused by the yeast-like Cryptococcus. The South American workers give the name "blastomycosis" to many fungus infections, ALMEIDA, for example, listing 108 different fungi as causing the disease. The authors have made no attempt to single out examples of GILCHRIST'S disease from the numerous case records reported from South America. They have found only two proved cases originating outside this country, one of which was described in Canada by SHEPHERD and RHEA and the other in England by DOWLING and ELSWORTHY. McKENTY and MORGAN reported four Canadian cases, one of which was fatal, but since no description of the organism was given, other than the statement that it was "definitely mycelial", these patients must be placed among the presumptive group. Another Canadian case was described by GILLIES with positive cultures but no description of the fungus was given. Other presumptive cases have been reported from the Philippines, Egypt, and Russia. Inadequately described cases have been reported from Canada, Puerto Rico, Costa Rica, Russia, England, China, South America, France, and Mexico. Of the 340 cases in which a statement is made as to the locality of origin of the disease, 92 per cent of all cases, 95 per cent of the proved and presumptive cases, and 98 per cent of the proved cases have originated within the United States. Skin- In systemic blastomycosis the skin lesions are usually multiple and widely disseminated which is in contrast to that seen in the typical cutaneous type of the disease. The lesions in the latter form are more commonly single and spread slowly and peripherally. Very extensive involvement of the skin without evidences of infection of the internal organs also can occur. The face is most often involved, having been infected in 49 of the 109 proved and presumptive cases, the lesions occurring most frequently on the cheek, eyelids, and nose.

Lungs- The lungs were found to be more frequently involved than any other of the internal organs of the body. Of the 60 cases of proved systemic blastomycosis, lung lesions were demonstrated by necropsy in 37, and infection of the lungs was suspected or diagnosed clinically in 12 additional cases. Of the 56 presumptive cases, lung infections were found by autopsy in 28 cases, and a clinical diagnosis was made in 16 patients. Heart- The heart was involved in 9 instances lesions having been found in the pericardium, myocardium, and endocardium. Skeletal system—The bones and joints were infected in 50 per cent of proved and presumptive cases. Of the 59 proved cases of systemic infection, 31 patients had bone or joint lesions at autopsy, and in 4 instances a clinical diagnosis was made, making a total of 35 (60 per cent) of proved cases showing infection of these tissues. The spleen was involved in 24 cases, as was the liver. The pancreas was infected in 4 instances. The kidneys were involved in 22 cases, 16 of which were proved to be blastomycotic. The gastrointestinal tract is only occasionally the site of a lesion. Infection of the peritoneum has been reported rarely. The adrenals have been invaded in 2 cases of proved blastomycosis (15, 215) and in one case presumably blastomycotic. The thyroid gland was involved in only 1 case, and invasion of the thyroid cartilage has been described. Lesions of the prostate have been reported in 11 patients, testis in 4 cases, epididymis in 3 cases, and the seminal vesicles in 2 patients. The first description of invasion of the uterus and tubes by *Blastomyces dermatitidis* was reported from the authors' hospital and is included in the present series. Blastomycotic lesions of the central nervous system have been found in 16 cases. Other unusual sites of infection are the eye and larynx. Among the presumptive cases they found a description of infection of the larynx and vocal cords and a history of a patient with retropharyngeal abscesses. In summary the organs of predilection of the fungus *Blastomyces dermatitidis* are the skin and subcutaneous tissues, lungs, bones, joints, and less commonly the central nervous system. Infection of the abdominal organs occurs frequently, but the lesions usually are not sufficiently extensive to be of any pathological significance. Utilizing the antibody content of the serum as an index of the extent of the infection, the complement fixation reaction has been of value in demonstrating the homogenicity of the antigenic structure of the fungi isolated from their cases. The serum from one of the patients also fixed complement with cultures of *Glenospora Gammeli*, *Endomyces capsulatus*, *Monosporium tulanense* and *Blastomycoides tulanense*, all of which are morphologically similar to *Blastomyces dermatitidis* and considered by their group to be synonymous. Skin tests were done by injecting intracutaneously 0.1 cc of a heat-killed vaccine prepared by washing off the yeast-like growth on blood agar and diluting 1:1000 by volume in sterile saline. Within fifteen to twenty minutes after the injection of this material, a punctate erythema appears around the injected area, but this reaction is not specific as it has been occasionally observed in patients with nonblastomycotic infections. The specific reaction is very similar to a positive tuberculin reaction, appearing within 12 to 24 hours and reaching a maximum in two to four days. The reaction may proceed to sterile abscess formation in very allergic patients. The procedures necessary for isolation and identification of *Blastomyces dermatitidis* can be summarized as follows: The material should be streaked heavily on blood agar plates and SABOURAUD's slants. Incubation should be carried out at room temperature and at 37 C. The cultures should not be discarded for two or three weeks since colonies of Blastomyces may not appear for two weeks. The organism on blood agar at 37 C. grows as a small, wrinkled, waxy colony not dissimilar from a colony of tubercle bacilli. Microscopically it consists of large, rounded, thickwalled cells, many of them budding and some showing rudimentary hyphae. On SABOURAUD'S medium at room temperature the fungus grows as a white mold with an abundant aerial mycelium. Microscopically numerous branched hyphae with lateral conidia and thick swollen chlamydospores may be seen. If transplants of the mold-like growth on SABOURAUD'S medium are made to blood agar at 37 C., the fungus again reverts to the crinkly, waxy yeast-like colonies described above. Mold-like colonies can be obtained by transplanting the waxy colonies to SABOURAUD'S medium and incubating at room temperature. Systemic infection with *Blastomyces dermatitidis* is highly fatal, reaching a mortality much higher than that of tuberculosis. Of the 59 proved cases of the generalized disease, 49 were fatal within five years. Of the 57 presumptive cases, 40 were known to be dead within the same period; one patient died six years after the onset of symptoms and another died after approximately seven years of illness. The duration of illness in the fatal cases, dating from the time of onset of the first symptom, is given in table 3. The mortality rate of all 117 reported systemic cases (proved and presumptive) is 78 per cent if the patients who were not followed are included, and 83 per cent if they are excluded. It is 92 per cent if those who had been followed for less than two years from the onset also are excluded.

TABLE III. *Duration of disease in known fatal cases of systemic blastomycosis*

	Proved	Presumptive	Total
Died within 6 months	13	7	20
Died 6—12 months.	22	10	32
Died 1—2 years	8	7	15
Died 2—3 years	4	1	5
Died after 3 years	2	3	5
Length of illness undetermined . . .	0	14	14

The high incidence of the disease in people of poor economic circumstances is strong evidence that general hygienic measures are of great importance in preventing infection and should be of value in treatment after infection has been established. Since the prognosis differs so greatly, depending upon whether or not the infection is systemic or confined to the skin, the therapy of each type should be considered separately. Cutaneous blastomycosis: since GILCHRIST described the rapid healing of a large skin lesion and subcutaneous abscesses in a Negro after the administration of potassium iodide, this drug has been used extensively in the treatment of all forms (cutaneous and systemic) of this disease, usually with marked improvement in the majority of cases. One of the patients with skin blastomycosis illustrates the harmful effect of iodides when given to an individual who is allergic to the infecting fungus. A skin test with the standard heat-killed vaccine (diluted 1:1.000) resulted in the formation of a sterile abscess at the site of injection. Two weeks after iodide therapy was started, this patient, who was previously afebrile, began to have irregular fever reaching 39.6 C. (103 F) and the tissues surrounding the lesions became liquefied, leading to a serious spread of the infected area. He also lost 3.3 kilograms in weight in one week. The fever disappeared after discontinuance of the drug, and desensitizing doses of vaccine were given. After partial desensitization was accomplished iodides were again administered, resulting in gradual but satisfactory healing of the lesions. Among the local measures which have been used, usually combined with oral iodide therapy, are: iodide of lead ointment, silver nitrate, tincture of iodine, bichloride of mercury, 1 per cent copper sulphate, phenol, cauterization with a hot iron, and freezing with carbon dioxide snow. Curettage of the lesions and X-ray therapy have been successfully used in a large number of cases. Potassium iodide has been used more extensively than any other drug in the treatment of the systemic form of the disease, but as can be seen from the data on prognosis, the results are very discouraging. In the authors' series, gradually increasing doses of potassium iodide were of no effect in cases 1, 2, and 3. In case 8 there was a rapid improvement within two weeks after therapy was begun. Cases 4, 5, and 6 may be cited as examples to illustrate the harmful effects of the drug. One patient (case 4) had a pulmonary lesion which had remained stationary for about five years, and iodide therapy was followed by a rapid spread of the lung lesion and death. Case 5 improved slightly for two weeks but died in spite of vaccine and adequate doses of iodine. Case 6 improved clinically for several weeks, but the roetgenogram showed simultaneous spreading of the lung lesion and the outcome was fatal. Vaccine therapy was used by CHRISTENSEN and HEKTOEN in 2 patients, one of whom responded to the first injection with a rise in leucocytes, but no effect on the white count or temperature could be noted after subsequent injections. The second case did not respond to this form of therapy. BOUGHTON and STOBER reported promising results in their patient in which the injections caused a rise in temperature and an inflammatory reaction around the lesions. On the other hand, DICKSON and GARR could observe no beneficial effect. It is apparent from the few cases in the literature that the favorable result expected in vaccine therapy has been the production of focal or generalized reactions. In contrast to this the authors tried to avoid such reactions by the injection of only subreacting doses. Using the skin test as a guide, dilutions as high as 1 : 1,000,000 (1 : 1,000 dilution of the orginal 1 : 1,000 skin testing material) were injected, beginning with 0.1 cc. of the diluted material and gradually increasing the dose every two or three days. In case of any slight local or general reaction, the quantity injected was decreased to the previous nonreacting dose. When the dosage of diluted vaccine reached 1.0 cc, increasing doses of a less dilute suspension were injected in the same manner. In this way it was possible after four to eight weeks' therapy, to demonstrate a decrease in sensitivity as shown by skin test. In none of these cases was a negative skin reaction obtained, but abscess formation to the skin testing material was abolished, and the area of erythema produced by the injection was greatly reduced. Iodides could then be safely administered to these patients. Healing of the pulmonary lesions under this form of treatment is well illustrated in cases 10 and 11. As a result of their experience with this disease, they routinely skin-test the patients with the standardized *Blastomyces vaccine* before any form of therapy is considered. Iodides are given cautiously and only if the patient shows a negative or weakly positive skin reaction. Iodides are distinctly harmful if the patient is allergic. The comparative morphological and serological studies of different strains of *Blastomyces dermatitidis* indicate that these strains are antigenically homogeneous, and a stock strain maintained on blood agar can be used as a source of skin-testing material, complement fixing antigen and therapeutic vaccine. The case reports of 347 patients analyzed in part I of this study were scattered among 175 volumes of 92 journals. The largest number of cases reported in one series was 26 and was published by D' Aunoy and Bevan, who described in detail only one patient. The 11 cases reported by different authors in volume 13 of the Archives of Internal Medicine, thoroughly studied by all methods available at that time, did not include data on the serological and allergic status of the patient. The case histories of our 13 patients have been placed in the following sequence to emphasize certain clinical, immunological, and therapeutic features. Cases 1, 2, and 3 illustrate the clinical picture of the terminal stages of systemic blastomycosis in which no type of therapy is effective. Case 2 had a high titre of serum antibodies (indicative of widespread infection), and skin tests showed a state of anergy. Case 3 had a weakly positive skin test, a high titre of serum antibodies, and died one month after admission although thoroughly treated with potassium iodide. Cases 4, 5, and 6 illustrate the rapid spread of the disease during treatment with iodides. The fourth patient in this series had a chronic pulmonary infection, presumably blastomycotic, for five years before admission. He improved slightly during the first three weeks in the hospital, but after establishing the diagnosis of blastomycosis and inaugurating iodide therapy, the disease spread rapidly and the patient died within four weeks. Cases 7, 8, and 9 illustrate the findings in patients who, when first seen, were in good phycical condition and theoretically should have responded well to therapy. Case 7, although infected for six years, had not become hypersensitive to the fungus and developed no antibodies. Case 8 improved rapidly with iodide therapy in the hospital and continued to improve for six months after discharge, at which time he was apparently well and iodide therapy was discontinued. After remaining in good health for a year he relapsed and died. Case 9 was inadequately treated with iodides, and, although he had a slowly progressive disease for four years, he did not become hypersensitive and developed no antibodies. Cases 10, 11, and 12 improved satisfactorily with the combination of iodide and vaccine therapy. Case 10 is of special interest in that this is the only surviving patient in their series who had a high titre of serum antibodies. She was moderately allergic and, after desensitization by vaccine treatment, responded well to iodides. The operative evacuation of the focus in the uterus and tubes was followed by the disappearance of the antibodies, and the subsequent absence of evidence of new lesions suggested that the infection was brought under control. She was still in good health four years after the onset. Case 11 illustrates a high degree of allergy without serum antibodies. Injection of desensitizing doses of vaccine followed by iodide therapy resulted in rapid healing of the lesions. The failure to develop serum antibodies after vaccine therapy is offered as evidence that the beneficial effect of the vaccine did not result from active immunization. Case 12 illustrates the harmful effect of iodides on the skin lesions of a markedly allergic patient. Fever, loss of weight and extensive spread of the lesions occurred after iodide administration, and improvement resulted only after the iodides were stopped and vaccine therapy instituted. Case 13 is of interest in that it was possible to study the state of allergy to the protein and carbohydrate fractions of the fungus. Partial desensitization was accomplished by the intravenous injection of the carbohydrate fractions. From a study of the recorded cases of blastomycosis and their own series of 13 cases, they have drawn the following conclusions: American blastomycosis (Gilchrist's disease) is a distinct clinical entity caused by a specific etiological agent, *Blastomyces dermatitidis*. Two clinical types of infection are recognized: cutaneous blastomycosis, which proceeds as a chronic or subacute ulcerating process and which usually responds to treatment with iodides or radiation, and systemic blastomycosis, a highly fatal disease, characterized by pulmonary infection and widespread distribution of lesions in the subcutaneous tissues, bones, joints, central nervous system and internal organs. The same fungus, *Blastomyces dermatitidis*, causes both types of infection. The disease is far more common in males, and the natural habitat of the fungus is evidently in nature, although proof of its existence in the enviromment has not been found. Antibodies can be found in the sera of patients who are heavily infected; they persist until death unless the infectious process is overcome or greatly reduced. Some patients develop a condition of hypersensitiveness to the infecting fungus, and this allergic state diminishes in the terminal stages of the disease. The degree of hypersensitiveness can be estimated by skin tests and can be materially reduced by the repeated injections of minute doses of a heat-killed vaccine. Potassium iodide is curative in some cases, but it is a dangerous drug to administer to patients who are allergic to the fungus. In systemic blastomycosis iodide therapy should be started only after the state of hypersensitiveness has been excluded by skin test or artifically reduced by therapeutic injections of vaccine.

De Almeida and Da Silva Lacaz (1938) called attention to the clinical similarity of *glandular tuberculosis* and *blastomycosis*. They investigated five cases considered as blastomycosis, but they demonstrated Mycobacterium tuberculosis var. bovis. The histopathologic features of nodular-glandular blastomycosis and glandular tuberculosis are very similar.

Conant (1939) presented a *laboratory study of blastomyces dermatitidis* Gilchrist and Stokes 1898.

A critical review of the literature revealed that the fungus of blastomycosis has been placed in the following genera: *Oidium*, *Cryptococcus*, *Zymonema*, *Mycoderma*, *Glenospora*, *Acladium*, *Blastomycoides*, *Endomyces*, *Geotrichum*, *Monosporium*, *Aleurisma*, *Torulopsis*, *Gilchristia*, and *Trichosporium*. Some of these genera were based on the budding yeastlike appearance of *Blastomyces* in pathologic material; others were based on the filamentous growth in culture. In many instances new genera were proposed without an adequate personal study of the fungus. Some investigators, however, proposed changes in a sincere attempt to comply with the International Rules of Botanical Nomenclature in that the genus *Blastomyces* had been previously created by Constantin and Rolland (1888) for *Blastomyces luteus* and could not be applied to a different group of fungi. In spite of this complication it would seem that confusion would be avoided if Gilchrist's organism remained as *Blastomyces dermatitidis* until some definite agreement could be reached.

1. A comparative study of several strains of Blastomyces dermatitidis Gilchrist and Stokes 1898 isolated from cases of blastomycosis at Duke Hospital and elsewhere has been made.

2. Fungi reported from cases of blastomycosis but described under a variety of names were compared with *Blastomyces dermatitidis* and found to be identical.

3. All the strains studied developed as yeastlike cultures on blood agar at 37° C. and prickly to filamentous cultures on Sabouraud's glucose agar at room temperature.

4. The filamentous form of the fungus could be reversed to the yeastlike form by subculturing to blood-agar slants at 37° C.

5. Intraperitoneal injection of mice with 1 cc. of a 1 : 200 saline suspension of the yeastlike growth proved pathogenic for this laboratory animal.

Straus (1940) reported on *Blastomycosis* of the *Esophagus*.

Since blastomycosis was first described by Gilchrist in 1894, several hundred cases have been reported in the literature. In only 3 of these has there been involvement of the esophagus. Stepherd and Rhea and Martin and

SMITH have reported 2 cases, respectively, in which esophageal lesions were demonstrated at postmortem examination of patients with systemic blastomycosis. VINSON, BRODERS and MONTGOMERY reported a case of esophageal involvement which was noted clinically and proved by the use of the esophagoscope together with biopsy; unfortunately, however, the patient died one year later and autopsy was not obtained.

Esophageal obstruction due to local blastomycosis is a rare condition. However, since it can occur it must be considered in the differential diagnosis of obstructive lesions, especially since in the barium esophagogram it may so closely simulate carcinoma.

It is a matter of speculation as to whether the lesion in the reported case spread from the esophagus to the skin or vice versa. However, since dysphagia was the first symptom, preceding the cutaneous lesions by about one and one-half years, and since autopsy revealed no involvement of contiguous cervical and mediastinal structures which might suggest an infiltrating invasion, one may be justified in assuming that the primary inoculation occurred in an esophageal abrasion and produced a local lesion which may be likened to a typical cutaneous lesion. The lesions of the face were probably caused by an inoculation of sputum containing the organisms into an abrasion in the skin.

In the 2 cases reported by SHEPHERD and RHEA and MARTIN and SMITH, autopsy revealed widespread metastases with marked involvement of the mediastinal lymph nodes, so that, while in their reports no reference is made as to the mode of invasion, it is likely that the esophageal lesions developed by an invasion of the disease from the involved mediastinal structures.

The lesion reported by VINSON, BRODERS and MONTGOMERY showed a roentgenographic and esophagoscopic picture similar to that in the case reported here. Their patient, however, had advanced bilateral pulmonary tuberculosis, and since autopsy was not performed one cannot be sure that the esophageal lesion was purely local.

CALLAWAY and MOSELEY (1940) discussed *primary cutaneous* GILCHRIST'S *disease*.

This paper is intended as a brief discussion of the primary cutaneous form of GILCHRIST'S disease or North American blastomycosis.

Because of the writings in dermatologic texts and other reports, it is thought by many that GILCHRIST'S disease is confined almost entirely to that region known as the middle western United States. Although GILCHRIST'S disease is essentially a disease of the North American continent, it must be realized that it is apparently more widely distributed than has been thought by some authors. There are case reports of this disease from at least twentyeight states, in regions that are so widely separated as California, New York and Texas.

Males are seemingly infected about five times as often as females. Of one hundred and sixty-six reported cases of cutaneous GILCHRIST'S disease, one hundred and thirty-five males were infected and thirty-one females. The infection has been reported in all age groups.

There are clinically two forms of GILCHRIST'S disease: first, the primary cutaneous form which is the result of the inoculation of the organism *Blastomyces dermatitidis* into the skin. The second form is that in which there is a systemic infection of the body by this organism and secondary cutaneous involvement occurs with lesions produced in a manner similar to that seen in cases of secondary cutaneous tuberculosis.

Verrucous tuberculosis cutis, bromoderma, iododerma, epitheliomas, syphilitic gummas, and cutaneous fungus diseases of etiology other than *Blastomy-*

ces dermatitidis may prove difficult to differentiate clinically from cutaneous GILCHRIST'S disease and here various laboratory methods will have to be called on as aids in diagnosis.

There are five important laboratory diagnostic aids.

The first method is that of the examination of freshly prepared smears of material obtained from the abscesses or purulent discharges.

The second method is that of the recovery of the organism by culture.

The third laboratory aid consists of skin testing the suspected patient with an intracutaneous test dose consisting of one-tenth of a cubic centimeter of a standard heat killed vaccine. This test may be considered quite analogous to the tuberculin skin test and the results are read similarly.

As a fourth laboratory aid they have also used a complement fixation test with some degree of success. This test will probably seldom have to be called on in cases of primary cutaneous GILCHRIST'S disease.

The fifth method is that of biopsy and pathologic study. The histopathology of cutaneous GILCHRIST'S disease is quite distinctive. Epidermal hyperplasia of an extreme degree is found. Suppurative and tubercle-like reactions are found in adjacent areas of the skin section. In the deeper parts of the corium tubercles with mononuclear cell infiltration and giant cell formation occur. The bodies of the *Blastomyces dermatitidis* are found in the giant cells. Abscess formation is also seen in the corium. In the nodular or gummatous lesions the histopathology is more like that found in tuberculosis.

There are no exact figures as to the mortality of primary cutaneous GILCHRIST'S disease, but the mortality must be quite low, as patients with infection of several years' duration are often seen in whom no ill effects are noted. It is known, however, that the disease may progress to the systemic form through spread by way of the blood stream or lymphatic system. With systemic GILCHRIST'S disease the prognosis is quite poor. The mortality rate is estimated as being between 80 and 90 per cent. With this in mind, certainly every effort should be directed towards early diagnosis of suspected skin lesions.

Potassium iodide and other forms of iodides have been the drugs of choice in the treatment of this disease for many years, GILCHRIST, in 1902, being apparently one of the first to give a report on its efficacy. In studies at Duke Hospital they have found that this drug is, however, not entirely without danger. In patients found to have a marked sensitivity to the organism, spreading of the lesions has been found to occur after administration of iodides. Preliminary desensitization with a vaccine prepared from the *Blastomyces dermatitidis* seems to prevent this. At present all cases are first tested by the use of an intradermal test dose of 0.1 cc. of a one to one thousand dilution of heat-killed *Blastomyces dermatitidis* vaccine. Then, depending on the degree of reaction present, desensitizing doses of the vaccine are given. An autogenous vaccine is preferable, but not necessary. They have observed as good results with a stock vaccine as have been observed in cases treated by desensitization with autogenous vaccine. After this, iodides are administered by mouth in gradually increasing dosage, but in amount not exceeding five grams of the drug given three times a day.

Local treatment of the lesions is also of value. X-ray irradiation, radium applications, curettage, cauterization, the use of carbon dioxide snow, iodides locally, copper sulphate compresses, iontophoresis with copper sulphate, the local use of gentian violet, and various other local forms of treatment have all been tried and have given varying succes.

SMITH (1941) gave an excellent survey on *Blastomycosis* and the *Blastomycosis*-like *Infections*.

The purpose in this paper is to present these more or less similar diseases as a group and to emphasize their similarity and the importance of their differentiation in practice.

Various immunologic phenomena have been observed in several members of the blastomycosis-like group of diseases. The allergic eruptions usually referred to as "ids" have been seen associated with sporotrichosis, coccidioidomycosis and moniliasis. One would therefore expect to find them occasionally in other similar infections.

Positive complement fixation reactions have been obtained by various investigators in several of these mycoses, but the reactions are not sufficiently specific to be of diagnostic value. The same appears to be true of precipitation and agglutination reactions.

The local reactions to intracutaneous injections of specific fungous antigens appear to be of some aid in diagnosis and are worthy of further study. The reactions are of the tuberculin type, appearing in from twenty-four to forty-eight hours. A positive reaction to sporotrichin almost always occurs in sporotrichosis, although the reaction occasionally occurs in other mycoses as well. A negative reaction, according to SULZBERGER, speaks with a degree of certainty for the absence of sporotrichosis. In cases of coccidioidomycosis, JACOBSON and many others have found the cutaneous test with coccidioidin to be specific and of definite diagnostic value.

There are several nonmycotic conditions which are especially likely to mimic the diseases of this group of mycoses. A group of gummas extending in linear arrangement may closely simulate sporotrichosis. Ioderma and bromoderma at times give a picture similar to that of GILCHRIST'S disease, and when such a lesion occurs from iodide therapy in the course of blastomycosis or coccidioidal granuloma it is quite confusing. Tuberculosis verrucosa cutis usually has a dry verrucous surface, but in the presence of secondary pyogenic infection it somewhat resembles mycoses of this group. Sinuses extending to the surface from infections of bone and other deep structures are often difficult to differentiate from mycotic infection. In many open sinuses there is so much pyogenic infection as to make the finding and isolation of fungi a difficult problem.

The so-called "pseudomycoses" may resemble the true mycoses. Indolent nodules, sinuses and ulcers caused by *Micrococcus myceticus* of Castellani or *Bacillus typhosus*, and deep-seated, bruise-like nodules of *Bacillus pyocyaneus*, mentioned by WEIDMAN, belong to this group. SMITH has seen 2 cases presenting deep infiltrations and sinuses due to *Micrococcus myceticus* which resembled sinuses of coccidioidal granuloma, although somewhat more acute in appearance

The prognoses of these diseases vary considerably. Blastomycosis of GILCHRIST usually does not become systemic, although it occasionally does, with corresponding darkening of the outlook. A good many cases are responsive to iodides and roentgen therapy, although many cases are resistant to all known forms of therapy. With the acute pulmonary form of coccidioidomycosis the patient almost always recovers, but in coccidioidal granuloma the response to treatment is usually poor and the eventual mortality high. JACOBSON, however, reported good results from intravenous injections of coccidioidin. Paracoccidioidal granuloma has the reputation of being a fatal disease. Chromomycosis, or chromoblastomycosis, usually remains localized, but CARRION has reported a case presenting metastatic lesions. Whereas some of the American cases have responded to iodide therapy, CARRION'S experience in Puerto Rico has been that they are difficult to cure. Moniliasis in systemic form offers a serious prognosis. Torulosis, since it usually sooner or later involves the central nervous system, is likely to be fatal. Sporotrichosis, if localized, usually responds to iodides; with deeper involvement the outcome is less certain. In the case of scopulariopsosis referred to, cure was brought about by iodides. Lesions of rhinosporidiosis must be thoroughly destroyed; otherwise they are prone to recur. Histoplasmosis, as far as the author was able to ascertain, always results in death.

TABLE I. *Geographic Distribution of Blastomycosis and Blastomycosis-like Infections (from Reported Cases)*

Blastomycosis	North America, especially central states of United States
Coccidioidomycosis	United States, especially the vally of California few cases in South America
Paracoccidioidal granuloma	South and Central America
Chromomycosis.	South America, West Indies, United States, Russia, Japan, Africa
Moniliasis (deep)	United States
Torulosis	Europe, United States
Sporotrichosis	Throughout the world
Scopulariopsosis (deep) . .	United States (Colorado), Europe, Africa
Rhinosporidiosis	India, Ceylon, Italy, North and South America
Histoplasmosis	United States, Central America, Isthmus of Panama

TABLE II. *Clinical Pictures of Blastomycosis and Blastomycosis-like Infections*

Disease	Description	Prognosis as to Life
Blastomycosis	Infiltration with verrucous surface, miliary abscesses, ulceration	Good
Coccidioidomycosis	1. Acute pulmonary infection (valley fever) 2. Coccidioidal granuloma: Verrucous lesion with miliary abscesses; subcutaneous abscesses and sinuses; involvement of bones, joints and viscera (except intestinal tract)	1. Good 2. High mortality
Paracoccidioidal granuloma	Furunculoid nodules beginning about the mouth; may be verrucous; extreme adenopathy resembling Hodgkin's disease; intestinal tract involved	Fatal
Chromomycosis	Elevated, verrucous, dark growths; mostly on legs	Good; does not usually become systemic
Moniliasis (deep).	Subcutaneous abscesses, ulcers, hyperkeratotic infiltration	Serious if systemic
Torulosis	Acneform pustules, granuloma-like ulcers, deep abscesses, subcutaneous nodules wit hout ulceration, involvement of central nervous system	Grave except in ca- - ses of localized involvement
Sporotrichosis	Primary ulceration and nodules along lymphatics disseminated nodules and ulcerations; occasionally visceral involvement	Usually good
Scopulariopsosis (deep) . .	Painful nodules	Good
Rhinosporidiosis	Pinkish polypoid masses beginning on nasal septum; skin involved by transplantation	Prone to recur unless destruction thorough
Histoplasmosis	Intermittent fever, splenohepatomegaly, anemia, leukopenia	Fatal

TABLE III. *Description of Usual Organisms in Tissue and Culture*

Disease	Organism	Appearance in Tissue	Appearance in Culture
Blastomycosis	*Blastomyces dermatitidis*	Rounded, encapsulated cell some with buds	Amount of mycelium varies with medium; grouped, large round chlamydospores; characteristic mycelium septate; raquets
Coccidioidomycosis	*Coccidioides immitis*	Rounded, encapsulated cell some with endospores	Coarse, septate mycelium, raquets, arthrospores and chlamydospores
Paracoccidioidal granuloma	*Paracoccidioides brasiliensis*	Rounded, encapsulated cell without distinct endospores, multiple small protoplasmic buds	Asci with spores inside and protruding; septate mycelium and chlamydospores
Chromomycosis	1. *Phialophora verrucosa* 2. *Hormodendrum pedrosoi*	1 and 2. Thick-walled, dark cells, some showing transverse fission; no buds	1. Septate mycelium, flask-shaped conidiophores; 2. Mycelium septate, conidiophores bearing branching chains of conidia
Moniliasis	*Monilia albicans*	Rounded, budding cells; hyphae may be present	Some mycelium, masses of conidia, chlamydospores; no asci
Torulosis	*Torula histolytica*	Large number rounded, encapsulated cells, some budding	Budding cells; no ascospores; no mycelium
Sporotrichosis	*Sporotrichum schencki*	Small, round or elongated spores, difficult to demonstrate	Profuse, fine mycelium; pearshaped, elongated spores in groups on short stalks
Scopulariopsosis	*Scopulariopsis brevicaulis*	Minute, encapsulated cells, difficult to identify	Septate, seldom branching, mycelium bearing long chains of conidia
Rhinosporidiosis	*Rhinosporidium seeberi*	Numerous, very large cystlike sporangia containing many spores	Not grown on artificial mediums
Histoplasmosis	*Histoplasma capsulatum*	Many very small cells with pale capsules, within endothelial and giant cell	Septate mycelium, conidia and chlamydospores; asci with ascospores and multiple tubercle-like buds

MARTIN and JONES (1941) reported on *systemic Blastomycosis* in a case with unusual immunologic findings, well two years after onset.

The treatment of blastomycosis (American form) varies with the individual patient, depending upon the extent of the infection, the degree of allergy developed by the patient, and the locality and nature of the individual lesions. The following case is reported because the patient presented an immunologic picture markedly different from that usually seen in blastomycosis, which necessitated a radical departure from the treatment usually used in this disease. After failure to obtain improvement with iodides, this treatment was supplemented by subcutaneous injections of small amounts of immune serum and the introduction of maggots into the local lesion.

The possible effects of these two additional agents in bringing about the ultimate cure of the patient are discussed.

JONES and MARTIN (1941) gave a review on *Blastomycosis* of *Bone*.

In reviewing the literature on generalized blastomycosis they have been able to collect 63 cases which showed evidence of bone involvement. Six of these patients recovered after treatment, 3 were not followed sufficiently long to determine their end result, and 53 died of the disease. The detailed case report accompanying this article records the history of a seventh patient with bone blastomycosis who was cured after prolonged treatment. Because of the usually poor therapeutic results, they believed it of interest to summarize the present knowledge of this type of infection and to outline certain studies which they have found valuable in the diagnosis, prognosis, and treatment of the disease.

In the majority of cases the course of the disease is that of a chronic febrile illness with progressive loss of weight and strength, a moderate secondary anemia, and leucocytosis. The pulmonary symptoms may dominate the picture, the patients having cough, varying amounts of sputum, hemoptysis, or pleural pain. Often there occurs progressive development of soft tissue abscesses with or without bone involvement. In allergic patients these abscesses may develop very rapidly, show signs of acute inflammation, and contain a bloody tenacious exudate. If the degree of allergy is less marked, the "cold" abscesses contain thick purulent material.

Distribution of bone and joint lesions in 63 *reported cases of generalized blastomycosis*

Location	No. of Cases	Location	No. of Cases	Location	No. of Cases
Vertebra [1]. .	25	Shoulder. .	1	Hip . . .	3
Skull	21	Elbow . .	7	Knee . . .	11
Ribs	19	Ulna . . .	4	Tibia . . .	16
Sternum. . .	8	Radius . .	2	Fibula. . .	6
Clavicle . . .	4	Carpus . .	10	Patella . .	3
Scapula . . .	2	Metacarpals	5	Tarsus . .	12
Pelvis. . . .	5	Phalanges .	3	Metatarsals	10
Humerus . .	4	Femur . .	5	Toes . . .	3

The clinical diagnosis of blastomycosis may be suspected from the history and the physical findings. However, a similar picture can be produced by infections with other fungi, tuberculous osteomyelitis, and tertiary syphilis of bone.

The isolation and identification of *Blastomyces dermatitidis* from infected secretions or exudates is proof of infection and should be attempted in all cases.

The skin tests are done with a heat-killed vaccine. This vaccine is prepared by suspending, in sterile saline solution, the yeastlike organisms from a culture of *Blastomyces dermatitidis* grown on blood agar at 37° C. This suspension is centrifuged in a HOPKIN'S tube and the sediment suspended in enough saline solution to make a 1 : 1,000 dilution by volume. The standardized suspension is heated to 60° C. for four hours and tested for sterility by heavily inoculating a blood agar slant and incubating at 37° C. for at least ten days. Tricresol, 0.35 per cent, is added as a preservative. This material is used for skin testing by injecting 0.1 cc. intracutaneously. Usually an area of erythema appears about the site of injection within fifteen to twenty minutes. This reaction is not specific and is found in patients having pulmonary disease of other types. The characteristic positive reaction, beginning after twelve to twenty-four hours and reaching a maximum in two to four days, resembles closely a positive tuberculin reaction. In very allergic patients a sterile abscess may form at the site of inoculation.

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[1]) Paravertebral abscesses were present in 14 cases.

The complement fixation test specific for *Blastomyces dermatitidis* has been described by MARTIN, and can be done in any laboratory equipped to do blood Wassermann reactions. The proportions of material and reagents are the same as used in the Wassermann test except for the substitution of a fungus suspension for the beef-heart antigen. Both of these procedures, i.e., the test for sensitivity to heat-killed *Blastomyces vaccine* and the complement fixation test, can and should be carried out as initial studies on patients suspected of having blastomycosis.

The skin test with *Blastomyces vaccine* serves not only as a diagnostic procedure but also as a means of determining whether or not the patient should be desensitized. If the vaccine produces a sterile abscess, desensitization should be started with a dose of 0.1 cc. of a 1:1,000 dilution of the vaccine (a 1:1,000,000 dilution of the original material). This is administered no more frequently than every other day and the dosage is kept below the point of causing a local or general reaction. Less sensitive patients can be started with a higher initial dosage. After the degree of allergy has been reduced, as judged by skin testing, iodides may be administered cautiously. They usually give a saturated solution of potassium iodide; beginning with 3 drops t.i.d. and increasing 1 drop per dose every day until tolerance is reached.

Potassium iodide was given to all six patients who recovered from bone blastomycosis. Three of these also received heliotherapy which was believed to be beneficial. Two patients were given *Blastomyces vaccine* with apparent benefit and in one case x-ray therapy was thought to have been responsible for the improvement. There is a recent report of a patient who recovered after treatment with potassium iodide, antimony, and potassium tartrate intravenously, and 3 cc. of colloidal copper intramuscularly every five days for twenty doses. The last drug was believed to have caused the patient's improvement.

The prognosis of the majority of patients having blastomycosis with bone lesions is poor, especially in those individuals who have a high titer of complement fixing antibodies.

SMITH (1941) gave an excellent summary of *Blastomycosis* and *the Blastomycosis-like infections.*

The clinical characteristics of blastomycosis of GILCHRIST and the group of other mycoses in which yeast-like bodies are found are often not sufficient for diagnosis. In some forms of the group the microscopic appearance of the organism may be characteristic and diagnostic but in most cases the study of cultures is necessary. An accurate mycologic diagnosis is important in view of the different prognoses and the varying degrees of therapeutic response of infections by these different fungi, and for the purpose of adding new members to the list of possible pathogens. Intracutaneous tests with fungous antigens offer some assistance in diagnosis but must not be considered absolutely specific.

BAKER (1942) reported on *experimental Blastomycosis in mice.*

A study was made of the effects on mice of intraperitoneal injections of living *Blastomyces dermatitidis*, killed suspensions of the same organism and a phosphatide fraction of this fungus; also a polysaccharide fraction was studied in rabbits. The following results and conclusions are recorded.

The mouse was preeminently suited to the experimental production of blastomycosis. The experimental disease was characterized by the continued response of polymorphonuclear neutrophils to the luxuriant growth of the fungus throughout the infected animal and by the necrosis of the blastomycotic masses. The lesions in mice consisted mainly of organisms, whereas the lesions in most infectious diseases consist mainly of the reacting cells of the hosts.

Repeated intraperitoneal injections of heatkilled *B. dermatitidis* were toxic for mice, and often lethal. This is thought to explain the final lethal effect in the experimental disease, in which masses of organisms and intermingled reacting cells became necrotic, and probably permitted the absorption of substances like those associated with the suspensions of the heat-killed organisms.

Heat-killed and living blastomycetes provoked similar cellular responses in the peritoneal cavities of mice.

Blastomycetic phosphatide repeatedly injected intraperitoneally into mice caused cells of the monocytic series to respond. This fraction is therefore not responsible for the polymorphonuclear response and the necrotizing effect related to the living organisms.

In rabbits, single intraperitoneal injections of blastomycetic polysaccharide produced, in the first few hours, sterile peritonitis, retrosternal lymphadenitis and remarkable changes in the blood stream consisting of leukopenia, lymphopenia and increase in the numbers of immature amphophils. These changes are similar to those which have been produced by polysaccharides from tubercle bacilli and pneumococci, as described by SABIN, JOYNER and SMITHBURN.

Numerous case reports on *blastomycosis* from all over the world reveal a keen interest in this disease. Reports were published by CAROL (1939) from Holland; by STOCHDORPH (1939) from Germany; by RICHTER (1939) from Tchechoslovakia; by BROCCHIERI (1939), VENTURI (1938), from Italy. From North America we have reports by SOLWAY, KOHAN and PRITZKER (1939) from Canada; by ROBERTSON et al. (1939), LEVIN (1939), MARTIN and SMITH (1939) from the United States. The latter is particularly a valuable contribution. These authors give a historical survey and compilation of all cases of blastomycosis published in North America up to 1939, altogether 347 cases, among them thirteen observations of their own. Based on this large material and on their own investigations they discuss the question of the source of infection, the distribution according to age, sex and race, the seasonal and geographical distribution, the avenue of infection, localization in the various organs, immunologic and allergic phenomena, the clinical picture, diagnosis, prognosis and therapy. From South America reports are presented by RAMOS, ORIA and PIO (1939), DE ALMEIDA and DA SILVA LACAZ (1939 a, b) from Brazil. MAZZA et al. (1939 a) reported the first case of blastomycosis from the Andes region, and MAZZA et al. (1939 b) from the Chaco region, in Argentina.

NINO'S (1938) monographic presentation about the *blastomycoses* in Argentina is as comprehensive as it is outstanding. The report encompasses 31 cases of this disease, observed up to this date in Argentina. Numerous illustrations are included. According to our present knowledge, the observations are divided in three groups: 1. the blastomycosis per se (granuloma cryptococcoides — "Torulosis" in the U.S.A., 2 cases); 2. the parablastomycosis (granuloma paracoccidioides, 15 cases); blastomycosis Gilchrist (4 cases) and histoplasmosis or Darling's disease (with no observation in Argentina); 3. the pseudoblastomycosis with a. granuloma coccidioides Posadas (4 cases), b. granuloma rhinosporidioides Seeber (4 cases) and c. granuloma chromomycoticum (chromoblastomycosis) (2 cases). Each of these groups is clearly described and thoroughly discussed. The clinical features of the various mycoses are particularly well presented. The geographical distribution of these cases reveals that no cases of these mycoses were up to now observed south from Buenos Aires and that most of the cases occurred in the northern provinces. The geographical occurrence of these mycoses presents another interesting feature. One is the relative frequency of paracoccidioidomycosis in Argentina and with no observation on the North American continent; the other is the relative frequency of histoplasmosis in North America, particularly in the United States — with no observation of this condition in South America.

A number of case reports on *cutaneous* and *systemic blastomycosis* was presented by VENTURI (1938, septicemia), DE ALMEIDA and DA SILVA LACAZ (1939*a*, skin), SOLWAY, KOHAN and PRITZKER (1939, systemic), RAMOS and ORIA (1939, hematology), DE ALMEIDA and DA SILVA LACAZ (1939*b*, tongue), BROCCHIERI (1939, skin) CALLAWAY and MOSLEY (1940, survey), MARTIN and JONES (1941, bone).

G. Torulosis

(*The European Type*)

D'AUNOY and LAFFERTY (1939) reported on *Torula meningitis* in a negro boy, seven years of age.

Meningitis, produced by the yeast-like organism, Torula, is comparatively rare. With a single exception all of the sixty-three cases thus far reported occurred in persons over ten years of age; the majority being between thirty and sixty. The only case afflicting a child, a girl three years and four months of age, was observed by BARLOW in Australia (1923). The case here presented occurred in a negro child and is incidentally the second case of *Torula meningitis* ever reported from the State of Louisiana.

Three cases of *Torula infection* of the central nervous system were investigated by MAGRUDER (1939).

Case 1, on admission, presented a clinical picture resembling intracranial tumor. In view of the changing neurologic findings and the results of the laboratory and x-ray studies, the diagnosis was extremely problematical. Unsuspectingly, it had been made by culturing the yeastlike organism from the spinal fluid. Through the error of regarding it as a contaminant, this evidence was discarded. As a result, not until craniotomy had been done was the correct pathologic process determined.

Case 2 was a case of torulosis in a patient with diabetes mellitus and lymphatic leucemia. The diagnosis was made by the unsuspecting finding of yeastlike budding organisms on microscopic examination of the spinal fluid. These were identified by further laboratory procedures as *Torula histolytica*.

Case 3 is one of torula infection of the central nervous system of one year's duration. There has been no treatment since discharge from the hospital on May 8, 1937. The patient is still doing her daily work with little inconvenience from the infection. LEVINE ¹), in his article, found the average length of the disease to be five or six months. The longest case on record lived five and one-half years.

On June 7, 1939, Case 3 was reported to be still alive and carrying on her usual work.

OWEN (1940) reported on *generalized cryptococcosis simulating* HODGKIN'S *disease*.

A case of widespread, generalized cryptococcosis (torulosis) occurring in a white man, 38 years of age, is reported that was originally diagnosed clinically and from histological sections from cervical lymph nodes, as lymphosarcoma, HODGKIN'S type, and so treated until acute symptoms supervened five years from the date of the original diagnosis. Instances like this are proof that biopsy

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¹) LEVINE, E. A.: Torula Infection of the Central Nervous System, Arch. Int. Med. 59: 667, 1937.

of a lymph node is not always sufficient to make a diagnosis, that in addition to the histological examination, cultures of the freshly removed node should be made.

WADE and STEVENSON (1941) reported on *Torula infection.* A case of Torula meningo-encephalitis in a housewife 39 years old is described. The history was of four months' duration. The lesions of the central nervous system were characterized by the presence of mononuclear phagocytic cells and giant cells. No other viscera were involved.

The organism recovered from the cerebrospinal fluid was pathogenic for mice, as evidenced by the existence of lesions at the time of death, or when killed, in 36 of 40 inoculated animals.

The lesions produced in mice were most frequent and extensive in the lungs but extensive lesions were also common in the brain and kidney. The liver and spleen were involved less frequently and the lesions were smaller. Large mononuclear cells were present in most of the lesions; many of these cells contained *Torulae.*

Attempts to establish meningitis or encephalitis without involvement of other organs failed, although lesions of the nasal mucosa near the cribriform plate often followed intranasal instillation of organisms.

STILES and CURTISS (1941) observed a case of *Torula meningoencephalitis.*

The case which is the subject of the report was somewhat unusual because of the fact that the etiologic agent was demonstrated early in the course of illness and because of the relative acuteness of the disease. Only three other reported instances of torulosis have been of less than a month's duration. In JOHNS and ATTAWAY'S summary [1]), published in 1933, 46 cases of torulosis were described of which only 31 had occurred in the United States. In discussing CABOT'S case [2]) MALLORY stated in 1934 that he had seen an unpublished manuscript in which approximately 80 cases of torulosis were mentioned. Since then about 15 additional cases have been reported. The diagnosis was established before death in only about half of these cases, but the disease was not even suspected in a considerable number until an examination of microscopic sections had revealed the true nature of the condition.

BINFORD (1941) reviewed the recent literature on *Torulosis of the central nervous system,* adding one observation of his own.

Invasion of the central nervous system by the yeastlike organism *Torula histolytica* has been known since von HANSEMAN in 1906 described small gelatinous cysts containing yeast cells which he observed in the meninges of a man dying of meningitis. STODDARD and CUTLER in 1916 collected the published case reports of this disease, reported two cases and discussed the cultural characteristics, the pathogenicity for laboratory animals and the distinguishing characteristics from blastomycosis and coccidioidal granuloma. The name *Torula histolytica* which they used has persisted although much doubt as to its histolytic action has been expressed. FREEMAN and WEIDMAN in 1923, and FREEMAN in his exhaustive monograph published in 1931, have added much to our knowledge of the disease. In the latter publication 43 cases were reviewed. LEVIN summarized the literature in 1937 and reported two cases bringing the total recorded cases to sixty. The clinical, etiological and pathological phases have been thoroughly described by these and other authors.

Since LEVIN'S summary, reports of fourteen cases have appeared; some of the data from these are found in table 1. In general the clinical and autopsy observations agreed with cases previously described. Brain tumor or tuberculous meningitis were most frequently simulated. Epileptiform seizures had been present for 20 years in the case observed by DE BUSSCHER and co-authors and 4 years in the case reported by CUDMORE and LISA. In the latter autopsy revealed a calcifying cortical cicatrix containing torulae. The severe form of generalized torulosis which LONGMIRE observed in a colored girl aged 4 suggests the widespread involvement sometimes seen in laboratory animals. While the disease is relatively rare the antemortem diagnosis of three cases in three years by MAGRUDER at the University of Virginia Hospital, Charlottesville, Virginia, is significant. Its recognition will probably become more frequent when phsicians consider it among the diagnostic possibilities of obscure cerebral conditions and laboratory workers learn to recognize this yeast. In the first case reported by MAGRUDER a culture of yeast from the cerebrospinal fluid was considered a contaminant. Biopsy obtained by an exploratory operation for brain tumor showed torulae. Of the other eleven case reports antemortem diagnosis was made only three times.

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[1]) JOHNS, F. M. and ATTAWAY, C. L.: Torula Meningitis: Report of Cases and Summary of Literature, Am. J. Clin. Path. 3: 459 (Nov.) 1933.

[2]) TORULA MENINGITIS: Lymphoblastoma, Hodgkin's Type [Cabot Case 20241], New England J. Med. 210: 1291 (June 14) 1934.

The case of a forty-one year old man with torulosis of the central nervous system is reported. With this case the total number of recorded cases in now 75. The diagnosis was established by positive guinea pig inoculation obtained from three specimens of cerebrospinal fluid and by finding torulae on direct examination and culture of a fourth specimen. The patient is alive one year after the onset of severe symptoms.

REEVES, BUTT and HAMMACK (1941) gave a survey of *Torula infection* of the *lungs* and *central nervous system* with the report of six cases with three autopsies.

Not only may the antemortem diagnosis of torula infection be difficult, but frequently it is not made. In his 60 collected cases, LEVIN found the condition diagnosed prior to death in 37 instances and at autopsy in 23 instances.

Often the onset of the disease is insidious with intermittently severe frontal headaches, but not infrequently it may be sudden with headaches more pronounced and associated with spells of vomiting. Dizziness, vertigo and stiffness and pain in the back of the neck are not uncommon symptoms, and visual disturbances, such as amblyopia, diplopia, strabismus, nystagmus and ptosis, are also reported rather frequently. Mental symptoms, including confusion, drowsiness, restlessness and delirium, have been described commonly. Ataxia and hemiplegia have also been noted.

The commonest physical symptom has been stiffness of the neck, with positive Kernig and Brudzinski signs. Fever, if present, is of only moderate degree, and the leukocyte count is usually not much elevated. Neuroretinitis and papilledema are frequently seen. The reflexes have been variably reported Occasionally the Babinski sign is obtained. Although occasional periods of remission have been noted, the course of the disease is usually downhill, and the patient ultimately becomes comatose and dies of respiratory failure. If the lungs are involved, the clinical picture may suggest tuberculosis or tumor. There is usually a cough, some pain in the chest, malaise and loss of weight, but little or no fever or night sweats.

In the cases reviewed by LEVIN, it is interesting to discover that prior to culture or autopsy, tuberculous meningitis was diagnosed in 10 instances, undesignated meningitis in 5, encephalitis in 7, tumor and abscess of the brain in 6 and 4, respectively, and psychotic dementia in 5. He reported involvement of the central nervous system alone in 30 cases and of the lungs in 9 cases and generalized involvement in 8 cases.

One to six months has been the usually recorded duration of the disease. In LEVIN's series of 60 cases, however, he found the duration of the illness to be one month or less in 3 cases, two months in 10, twenty-five to thirty months in 5 and six years in 1.

In 1925 SHAPIRO and NEAL collected a total of 15 cases of torula infection, including 1 of their own. In 1930 FREEMAN reviewed the gross and histopathologic changes observed in 19 cases of torulosis and in his monograph in 1931 reported a total of 43 cases. Since that time LEVIN found 15 more which, with the 2 he reported in 1938, brought the total to 60 cases of invasion of the central nervous system by *Torula*. Including the cases recently reported by TABER, CALDWELL, CRONE, QUODBACH, CUDMORE and LISA, DE BUSSCHER and his associates, GREENFIELD, MARTIN and MOORE, LONGMIRE and GOODWIN, MAGRUDER, D'AUNOY and LAFFERTY and ROBERTSON and his associates, together with the cases reported here, the total number is now 79. In view of the early misleading reports on the identity of such fungous infections, however, one is forced to be somewhat hesitant about accepting the accuracy of the number of these cases reported in the literature to date, but their occurrence has at all events been relatively uncommon.

In spite of various efforts at treatment, none has proved effective, and in all proved cases of involvement of the central nervous system the disease has always progressed to a fatal termination. The fourth case is of interest because there has been continued improvement in spite of a duration of the illness of more than two and a half years. The sixth case represents, we believe, a generalized type of the infection.

The noticeable variation in the duration of torula meningitis must be related in some way to a difference in the general resistance of the host, as well as to a variation in the virulence or type of the fungus.

STILES and CURTISS (1941) reported a case of *Torula meningoencephalitis* with special emphasis on the observation of the cerebrospinal fluid. The fatal case of torulosis, occurring in a man, a manufacturer of leather, aged 63, was somewhat unusual because of the acuteness of the disease and because of the fact that the etiologic agent was demonstrated early in the course of the illness.

The changes in the cerebrospinal fluid may be summarized as follows: The total number of cells was less than 600 in every instance except one, the majority being lymphocytes. The percentage of polymorphonuclear cells occasionally reached 55. (The number of leukocytes in the blood was, as a rule, slightly increased.) The pressure was increased in most instances, but only exceptionally was it above 450 mm. of water. Values for protein were usually over 40 mg. per hundred cubic centimeters of cerebrospinal fluid. The majority of the sugar determinations were under 40 mg. per hundred cubic centimeters. Values for chloride ranged between 460 and 760 mg. per hundred cubic centimeters. A composite gold curve approached 2333321100, although the individual curves varied considerably.

The various microscopic and chemical determinations in the cerebrospinal fluid of our patient resembled those reported in other cases of torulosis. The five determinations of sugar, with values ranging between 43 and 59 mg. per hundred cubic centimeters, were somewhat higher than the average, but this finding may possibly be explained as due to the administration of large amounts of sugar.

MARSHALL and TEED (1942) reported on *Torula histolytica meningoencephalitis* with recovery following bilateral mastoidectomy and sulfonamide therapy. The patient was a white girl, aged 9 years.

At the first examination it was felt that this was a relatively simple case of masked, bilateral mastoiditis with localized meningitis. The cerebrospinal fluid showed large numbers of yeast cells on direct smear of the centrifuged sediment. These were doubly refractive budding forms which have been identified as *Torula histolytica*. The finding of torulae came as a distinct surprise and called for a complete reevaluation of the case. It was thought that the torulae might have reached the meninges via the mastoids, but this opinion had to be abandoned in view of the complete absence of the yeast in either culture or histologic study of tissue from the mastoid. Consequently it came to be believed that the mastoid infection was a purely coincidental affair, possibly antedating but having little or no influence on the meningoencephalitis. The history of deafness was interesting, but after healing of the mastoid wounds tests showed practically normal hearing. The decided changes in the child's mental reaction following sulfadiazine therapy were most interesting, as was the regressive change following the discontinuance of the drug.

Evidence that mycotic infections as a class may be treated successfully by the sulfonamides is offered by POULTON, WALKER, BAILEY, MILLER and FELL, DORLING and ECKHOFF, OGILVIE and DOBSON, HOLMAN and CUTTING, in whose cases complete recovery from abdominal actinomycosis followed sulfonamide therapy. Several of the patients had been treated by the usual methods, such as surgery, iodides and roentgen rays, and were losing ground. Healing promptly followed the institution of sulfonamide therapy. Recovery from actinomycosis involving the cervicofacial region and thorax has been reported by DOBSON, HOLMAN and CUTTING, MORTON, SUDLER and JOHNSON and JORGE, BROWZE and MEALLA.

In addition, HEMMENS and DACK reported cure in rabbits of otherwise fatal experimental infections with *Bacterium necrophorum*, a close relative of the *Actinomyces*, following sulfonamide therapy. VAN BREE reported recovery in a case of moniliasis, SCHROEDER in one malignant blastomycosis and DIXON in one of *Madura foot (Actinomyces madurae)* as a result of the same treatment.

WARVI and RAWSON (1942) reported)a case of *fatal torula meningitis* complicating HODGKIN'S disease in an American-born white man, aged 39.

The characteristic features of this case of *torula leptomeningitis* are the insidious onset and the intermittent but progressive course, going on to a fatal termination. The increasing intracranial involvement caused the following consecutive manifestations: severe headaches; visual, sensory and motor disturbances; weakness; projectile vomiting; disorientation; coma, and respiratory failure.

The interesting features of this case are the occurrence of the meningitis as a complication of HODGKIN'S disease and the effect of the roentgen ray treatments to the head. This is the eight reported case of associated HODGKIN'S disease and torula meningitis. The marked reduction in cells in the cerebrospinal fluid is the only positive evidence of the effect of roentgen therapy, but it does not indicate whether it was harmful or beneficial except that the patient survived eighteen months after the first severe symptoms; for the most part he was ambulatory and in relative comfort. The usual course is one of severe symptoms and death within six months.

A review of the literature indicates that a diagnosis of torulosis is to be suspected in cases of otherwise unaccountable progressive intracranial disase. Confirmation is obtained by studies of the spinal fluid, with identification of the organism either by culture or by animal inoculation. A biopsy of representative tissue from the meninges will disclose the specific lesions.

The only undoubtedly beneficial therapeutic procedure appears to be repeated withdrawals of spinal fluid to promote drainage and decrease intracranial pressure. The use of acriflavine hydrochloride intrathecally may offer some possible curative effect in cases of this invariably fatal disease.

Numerous case reports were also presented on infection caused by *Torula histolytica:* ROBERTSON, ROBERTSON, RIGGS, and SCHWARTZ (1939, cerebrum), MAGRUDER (1939, central nervous system), GOLDBERG (1940, central nervous system), WADE and STEVENSON (1941, meningo-encephalitis, animal inoculation).

H. Chromoblastomycosis

DE ALMEIDA surveyed (1940) the present status of the question of the chromoblastomycosis in Brazil. In another publication (1941) he investigated the fungi as causative organisms in chromoblastomycosis. He studied different strains of the genus *Phialocondiophora* and came to the conclusion that it represents a valid genus. The occurrence of this genus was established in Sao Paulo, Minas Gerais, Paraiba and Para.

EMMONS et al. (1941) reported the sixth case of *Chromoblastomycosis* from continental United States. The etiologic agent was *Hormodendrum pedrosoi*. The case is interesting because of the early diagnosis (the case was of three months' duration), the unusual location of the lesion (wrist) and the apparently good response to potassium iodide therapy.

I. Coccidioidomycosis

COURVILLE and ABBOTT (1938) reported on the *pathology of coccidioidal granuloma of the central nervous system and its envelopes.*

Like most of the other granulomas, coccidioidal granuloma, a disease especially common in California and provoked by *Coccidioides immitis*, presents a wide variety of lesions of the nervous system and its envelopes. Lesions of the skull are usually responsible for small localized extradural abscesses, while in the spine an extradural abscess following disease of the vertebra may assume such proportions as to cause an extensive compression of the spinal cord. Subdural lesions have not been described. Leptomeningeal lesions in particular present a wide variety of appearances. The characteristic lesion is a small flattened tubercle with an irregular margin which is found over the dorsolateral surface of the brain in the vicinity of the branches of the middle cerebral artery. In this case, the disease may be mistaken for tuberculous meningitis. A diffuse granulation tissue may be found in the basilar cisterns, also similar in this respect to tuberculous meningitis. Modifications of these subarachnoid lesions are the cisternal abscess, usually involving the cisterna magna, and localized subarachnoid granulomas, which in the case of the spinal cord may be mistaken for a spinal cord tumor. Lesions of the nervous tissues themselves are relatively rare and consist of small circumscribed, somewhat irregular accumulations of granulation tissue. Such granulomas may be found in the cerebral centrum or central nuclear masses. They may also occur in the cortex although such examples are unusual.

MCMASTER and GILFILLAN (1939) reported on *Coccidioidal osteomyelitis.*

The surgical and pathologic aspects of bone and joint involvement in coccidioidal granuloma have received little attention in published articles. Twenty-four such cases have been studied and form the basis of this report. The condition is quite often misdiagnosed as either a pyogenic or a tuberculous lesion.

WERNICKE reported the first case of coccidioidal granuloma in 1892 from South America. RIXFORD and THORNE reported two cases from California in

1894 and two years later RIXFORD and GILCHRIST gave a detailed report of these two cases and suggested the name *Coccidioides immitis* for the organism, which was later termed *Oidium coccidioides* and the disease coccidioidal granuloma by *Ophuls* in 1905. The exact classification of the organism is still unsettled. The North and South American strains of the disease have been presented by JORDON and WEIDMAN.

Numerous reports dealing with the condition to 1931 are listed by CARTER in a review of the subject and a discussion of the roentgenographic diagnosis.

Twenty-four patients with coccidioidal infections proved by demonstrating the organism either in culture or by microscopic section of bone and joint have been studied, of whom two were women and the others men. The average age of the patients was 32 years. Thirteen of the twenty-four patients died, and each had an associated active pulmonary focus. Ten of these thirteen fatal cases showed multiple osseous lesions, while three presented only one demonstrable bony focus. The eleven patients who survived were studied from a few months to seven years after discharge from the hospital, and only two of them had an infection of the lung; in both instances this was not marked and underwent healing. Five of the patients who survived had multiple bone or joint infections, while six had only a single lesion.

There were four patients in the series younger than 20 years, of whom three survived.

Cutaneous infections, excluding those of draining sinuses overlying osseous infections, occurred as nodes or papules in six cases.

Four of the twenty-four cases studied exhibited primary localization in synovial membranes while the remainder were initially osseous in location. Some of the latter cases gave rise to joint infections by direct extension. Of the four cases of primary infection of the synovial membrane, one was of the ankle and secondarily invaded the ankle bones. Another was of the hip joint, giving rise to a spontaneous dislocation. The other two were of the knee joint.

Only four of the twenty-four cases occurred in children with open epiphysial lines. One of these four cases involved primarily a radial metaphysis while another occurred in a radial epiphysis. A third case exhibited initial localization in both a lower radial and a lower tibial epiphysis. The remaining infection was primary in the synovial membrane of the hip joint.

The roentgenographic picture is somewhat dependent on the stage and virulence of the disease. Bony foci in the early and acute stages are primarily destructive, with hazy outlines, little if any surrounding bony production and usually no demonstrable periostitis.

The more common chronic form of coccidioidal infection of bone exhibits a destructive type of lesion, with associated bone production and more often than not an overlying ossifying periostitis. Some of the osseous lesions will show an irregular margin with variable amounts of new boneformation. Others, however, will present a fairly sharply outlined wall about a cystlike cavity, being slightly suggestive of a bone cyst. At times these spaces may even show a tendency to trabeculation, giving a multilocular appearance.

The coccidioidal foci of bones of those patients who have died of the rather acute form of the disease show grossly necrosis and abscess. There is little if any evident production of tissue in the surrounding bone. Cortex may be partially destroyed with an associated abscess of the soft tissue. Overlying periosteum may appear edematous and thickened or partially destroyed.

The osseous lesions of the chronic type of the disease reveal either an abscess or a cavity filled with soft to firm granulation tissue or spongy bone with interstices filled with grayish granulation tissue. Bony "sand" is sometimes palpable in the regions of liquefaction necrosis. Occasionally larger sequestrums are seen. The surrounding bone may appear either hyperemic or irregularly sclerotic, and at times the region of destruction will appear cystic with varying degrees of bony sclerosis of the walls. The cortex overlying a cancellous lesion may be destroyed and an adjacent abscess in the soft tissue or a granulomatous mass noted. Adjacent to the region of cortical destruction a thickened periosteum is noted, which quite often will show ossification. These same periosteal reactions may be present overlying cancellous lesions, even though there is no apparent erosion of cortex.

Examination of infected joints shows a grayish thickened synovial membrane. Free fluid ranging from serous to seropurulent is also noted in some cases. Articular cartilage reveals marginal erosion by granulation tissue, which also usually involves articular cortex, and pannus may be seen. Segments of thinned articular cartilage may be separated from underlying cortex by hyperemic granulation tissue.

The treatment of coccidioidal infections of bones and joints is of a general as well as of a local nature. If the patient has an associated active pulmonary lesion, this must receive special attention, for all such patients in our series died except two who had minimal pulmonery foci. General supportive measures as well as rest and immobilization of the involved bone or joint lesion are indicated. The latter measures are requisite to prevent metastatic spread of the disease, especially since the disease has a tendency to multiplicity of foci.

Chronic infections in which bone abscesses are present should be treated by surgical drainage with cauterization of the area by removing the surrounding infected bone. The cavity is packed loosely with petrolatum gauze to allow for granulation from the bottom and the part immobilized in plaster of paris casts after the method of ORR.

Cures of infected joints appear to result only from ankylosis. Amputations were performed in three cases, one of which was for an infected large toe and two were for diseased ankles. All three patients survived but none had active lung lesions. Since the mortality is rather high in this disease and the chance of multiple foci developing is common, removal of a peripheral, well advanced bone or joint infection by amputation should be considered and is at times indicated. In addition to either conservative or surgical procedures in the treatment of bone and joint infections, whether single or multiple, general supportive measures and usually absolute bed rest are necessary.

RUDDOCK and HOPE (1939) reported on *Coccidioidal peritonitis* diagnosed by *peritoneoscopy*.

This case is unique in that it is the first case of coccidioidal peritonitis reported in the literature in which a diagnosis was proved before death.

The similarity of this disease to tuberculosis is noted repeatedly in the literature. Coccidioidal infections often are miliary and it is impossible without a biopsy or culture of the organism to differentiate them clinically or by roentgenogram from tuberculosis. The X-ray appearance of bone lesions is identical with the appearance of lesions caused by the tubercle bacillus.

The following case report demonstrates the value of peritoneoscopy in determining intraperitoneal disease, and the value of obtaining a biopsy.

History — Y.S., a man aged 35, Japanese, single, entered the hospital Dec. 23, 1937, complaining of swelling of the abdomen and pain in the abdomen of two weeks' duration. Two weeks before entry he began to have pain in the epigastrium. With the onset of pain his abdomen had begun to swell and was progressing in size. Loss of weight was slight. He did not have fever or chills. He did not vomit but had occasional nausea; no hematemesis or melena was present. There had been no symptoms of cardiac or respiratory disease. No jaundice was observed.

The patient was born in the Hawaiian Islands; he had lived in and about Los Angeles for the past eleven years. The patient stated that he had not resided in the San Joaquin Valley. He had a lesion of the penis in 1936 for which he received no treatment. For the past four years he had been a heavy user of alcohol, drinking both beer and whisky.

The clinical diagnosis was atrophic cirrhosis (LAENNEC). December 24, paracentesis was done and 4,000 cc. of straw colored fluid was removed. The patient continued to have a low grade fever and to develop more ascites. December 30, peritoneoscopy revealed dense adhesions of the omentum to the epigastric peritoneal surfaces. All peritoneal surfaces were covered with miliary tubercles. The liver and gallbladder were normal. A section of peritoneum containing tubercles was removed for biopsy. The abdominal cavity was filled with oxygen. The impression was probable tuberculous peritonitis, but final diagnosis awaited the result of biopsy. The pathologic examination of the tissue revealed tuberclelike nodules containing *Coccidioides immitis*. Culture of the peritoneal fluid yielded *Coccidioides immitis*. Immediately following peritoneoscopy an x-ray examination of the chest was reported negative. The patient died January 25.

The anatomic diagnosis was (1) coccidioidal granuloma; (2) coccidioidal lymphadenitis, generalized; (3) coccidioidal peritonitis, generalized; (4) coccidioidal pleuritis, bilateral with effusion; (5) coccidioides of spleen; (6) coccidioides, miliary, of liver; (7) coccidioides of left kidney. The clinical course with the development of ascites and a history of alcoholism supported a diagnosis of cirrhosis. The conditions as noted with the peritoneoscope were similar to those of tuberculosis of the peritoneal cavity, and oxyperitoneum was instituted as a treatment procedure. Differential diagnosis between tuberculosis and coccidioidal granuloma is not possible without biopsy or culture. A biopsy of the parietal peritoneum and a culture of the fluid revealed *Coccidioides immitis*, thus establishing the diagnosis.

THORNER (1939) observed cases of *erythema nodosum in childhood associated with infection by the Oidium coccidioides.*

The occurrence of erythema nodosum in these seven cases seems to coincide with the arrival of maximum sensitivity or allergy to the *Oidium*, at which time it is believed that the fungus has undergone the necessary change within the tissues from the vegetative phase (chlamydospores) in which it is inhaled, to the mature endosporulating state in which it is recovered in sputum and stomach washings.

Seven cases of erythema nodosum, definitely proven to be associated with the *Oidium coccidioides* in children ranging from the ages of three and one half to thirteen years, have been presented as being independent of either tuberculous or rheumatic infection. Only those specimens in which the fungus could be cultured on SABOURAUD's medium, recovered from guinea pigs after inoculation and then recultured, have been classified as "positive". In all instances a definite pulmonary complication accompanied the erythema nodosum as shown by clinical and x-ray findings, but which resolved entirely within a month's time and failed to progress later into granulomatous or caseous lesions. No tubercle bacilli were ever isolated from any of the specimens and in all but one case, the Mantoux tests were routinely negative. In most instances some direct contact with dusty areas or activities had preceded the illness by a few weeks, permitting the inhalation of a greater number than normal of the chlamydospore-containing mycelia from the surrounding dust, and which would bear out OPHUL's original contention that the primary infection with *Coccidioides immitis* in the majority of cases occurs through the respiratory tract. The natural habitat of the fungus has not been accurately established as yet because of the difficulty in successfully isolating the *Oidium* from the soil on culture medium, due to overgrowth of contaminating fungi and bacteria, although one such successful culture has been reported.

STORTS (1939) observed a case of *Coccidioidal granuloma simulating tumor in a child of four years.*

The following case report is of interest because of the relative rarity of coccidioidal granuloma and because it simulated brain tumor.

M. E. H., a girl aged 4 years, first seen October 4, 1937, had been "losing ground" for six months and had had headaches for four months, nausea and vomiting for two months and an unsteady gait for one month. The family history was not important. She had measles at 2 years and a tonsillectomy at 3.

The headaches had been periodic and mostly frontal. The vomiting had been frequent, usually in the mornings. The unsteadiness of gait, with the child staggering to the left, had grown progressively worse. She had occasional pains in both legs. Mentally she had been alert.

An exploratory operation disclosed a large cyst in the cisterna magna, with thickened arachnoid. The posterior wall of the cisterna magna, which is ordinarily covered by arachnoid of a spider web transparency, was covered by a white opaque membrane about 1 or 2 mm. thick. This entirely dammed back the spinal fluid and caused a large cyst.

Microscopic studies of the membrane revealed lymphoid infiltration and numerous concentric masses of cells. The periphery of these concentric masses was composed of dense, almost hyalinized, fibrous tissue, and the center was occupied by a large foreign body giant cell or a group of epithelioid cells. Within the body of the giant cells and in the crevices of some of the epithelioid cells there were spherical bodies with a double contoured highly refractile capsule. The diagnosis was coccidioidal granuloma.

The child was somewhat better for several weeks after the operation. However the course of the disease continued to be hopeless, and the child died.

An autopsy performed revealed a recurrence of the membranous lesion at the base of the brain and the formation of fibrous adhesions to the brain substance. The cerebellum had been pushed down into the area surrounding the foramen magnum. The lower portion of the upper lobe of the right lung contained a small hard nodule consisting of caseous material and simulating GHON tubercle. This single, sharply circumscribed nodule, 1.5 cm. in diameter, was the only lesion in the lungs. Sections showed coccidioidal tubercles and *Coccidioides immitis.*

She had lived her entire life in Tucson, Arizona, and her case is the first reported in which the disease originated in this locality.

COX and SMITH (1939) investigated cases of *arrested pulmonary coccidioidal granuloma.*

Four cases of completely arrested coccidioidal granoluma of the lungs or of the bronchial lymph nodes in man are reported. On none of these were the granulomatous lesions a factor in the cause of death. Similar arrested lesions could be produced at will by injecting small numbers of spherules of a virulent strain of the fungus coccidioides intravenously into white rats or guinea pigs. The minimum fatal inoculum for rats inoculated in this way was approximately 10,000 spherules. In nearly all the arrested experimental lesions organisms remained viable for periods up to two and a half years. In a human case organisms were cultured fifteen years after they had been first demonstrated. It is concluded that instances of arrested coccidioidal granuloma are more frequent than has heretofore been realized, that they probably outnumber the fatal cases of this disease and that they are not restricted to the region of the San Joaquin Valley, Calif. The relationship of arrested coccidioidal granuloma to the benign clinical disease known as "valley fever" is as yet unknown. Although the lesions present a histologic picture of inactivity, they may contain viable fungi for many years and constitute a possible source of dissemination.

FABER and SMITH (1939) were concerned with *acute coccidioidomycosis with erythema nodosum in children.*

Coccidioidal granuloma, a chronic progressive disease affecting skin, bones, lungs, and meninges, has been known to the medical world since 1892. It has a mortality of about 50 per cent. The causative agent, the fungus *Coccidioides immitis*, has been recognized since 1896. That the same fungus is also the cause of an entirely different clinical syndrome with an acute course ending in complete recovery in practically all cases was not clearly proved and brought to the attention of the medical profession until 1937 when DICKSON published the first of his careful studies on the subject, one of them in collaboration with GIFFORD. To this syndrome DICKSON has applied the term "primary or acute coccidioidomycosis" to emphasize its differences from the chronic disease.

The clinical picture of the acute disease has long been known to physicians of the San Joaquin Valley under such names as "valley fever", "San Joaquin fever" and "desert fever," and one of its common manifestations, erythema nodosum, is familiar to the laity of that region as "the bumps". The acute disease, unlike coccidioidal granuloma, is common among children, occurring even during infancy, but has not hitherto been described in the pediatric literature. The present report is based on 24 cases, personally studied, in children under 15 years of age.

The coccidioides fungus occurs in two forms or cycles: one, the vegetative form which is found in culture and presumably in nature; the other, the parasitic form, which is found in infected tissues. There is good reason to suppose that the disease is as a rule spread by dust contaminated with chlamydospores and inhaled.

Coccidioidal disease is largely confined to the San Joaquin Valley, the southern component of the great central valley of California, although sporadic cases are also known to occur in Arizona. Another endemic center exists in Argentina, and JACONO isolated coccidioides from two Neapolitan silo workers who had never been outside Italy.

The known foci in California and Arizona have in common a semi-arid climate where irrigation is necessary. As DICKSON has pointed out, the fungus is found where the winters are relatively warm, rainfall sparse, and summers hot and dry with much dust. Although patients develop coccidioidomycosis throughout the year the incidence is least during the rainy season and greatest in the late autumn and early winter before the rains. Of the 24 cases, 3 began in the spring, 7 in the summer, 12 in the autumn and 2 in the winter.

No age is spared. In DICKSON'S questionnaire, 3 per cent of the patients were under school age and 18 per cent were of school age. GIFFORD, BUSS, and DOUDS found 26 per cent of their 104 patients with "San Joaquin Fever" to be under 20 years of age. They also pointed out that while the age distribution of these patients corresponded to that of victims of coccidioidal granuloma females outnumbered males by 2 : 1 (the same figure as that obtained by DICKSON) while with coccidioidal granuloma males outnumbered females by 4 : 1. In the present series, however, of 22 patients with erythema nodosum, 12 were girls and 10, boys. The youngest patient was 15 months old at the time of onset, and the disease has been noted in a person of 69 years.

Recent arrival in the region was found to be an outstanding predisposing factor. Of the 24 patients 9 had lived in the San Joaquin Valley less than a year.

The portal of entry is almost certainly the respiratory tract. The fungus is probably conveyed in dust, and the frequency of cough and sore throat at the onset points to an initial respiratory infection.

The acute primary infection with fungus coccidioides has the features of a systemic infection, not unlike influenza, with both upper and lower respiratory manifestations. Because of the general nature of the symptoms at the onset the patient is rarely brought to the physician then or, if he is, the physician usually makes a diagnosis of influenza, pleurisy, or occasionally bronchopneumonia. However, in the San Joaquin fever form of the disease, after an interval during which the patient often feels well, the undiagnosed first stage is followed by a spectacular second stage marked by erythema nodosum and sometimes arthritis and conjunctivitis which brings the patient to the physician and enables him to make the correct diagnosis. This is the classical picture of valley fever seen in nearly all the patients of the present series and in the great majority of primary infections which have been diagnosed up to the present time.

A review of the cases has revealed the following picture.

The onset was usually sudden with moderate fever and in most cases a dry cough. Occasionally there was a chill. In about half the cases generalized aching, sore throat, headache and loss of appetite were each noted; and one or more of these in nearly every case. Rarely the children were merely fretful. In more than a third of the cases there was pain in the chest, either unilateral or substernal, which was made worse by deep breathing and was obviously of pleural origin.

These initial symptoms subsided in a week or so, but were occasionally more persistent. In three instances an erythematous rash, generalized or limited to te trunk appeared a few days after the onset. Night sweats also occurred at about the same period in three cases.

At an interval ranging from three to eighteen days with an average of eleven days from the onset, erythema nodosum appeared in 22 of the 24 patients. In all cases but one the lesions appeared on the anterior tibial surfaces and were fairly symmetrical. In many instances they also appeared on the lower anterior surfaces of the thighs, occasionally on the lateral surfaces of the hips, thighs and legs, on the buttocks and on the extensor surfaces of the arms forearms and elbows. The lesions presented the typical appearance of raised, brightly erythematous, roughly circular patches, sometimes confluent, tender to pressure, which gradually turned to a purplish and eventually to a brownish color and disappeared in the course of one to three weeks. In a few instances the lesions recurred after a few days or came out in successive crops. During this period fever usually recurred; as a rule it was low, but in one case reached 103.5° F. Simultaneously with the erythema nodosum, signs of acute arthritis were noted in 9 cases, involving the knees and ankles and in 1 case the shoulders. The joints were not red but were tender to pressure, painful on motion, and in one case slightly edematous. Hyperemia of the conjunctiva was also noted at this time in four cases. In one case erythema multiforme appeared at the same time on the forehead, cheeks, and neck.

In the San Joaquin Valley it is possible to make a presumptive diagnosis of primary coccidioidomycosis on the basis of an attack of fever and cough with one or more of the other onset symptoms such as generalized aching, headache, loss of appetite, sore throat, and pleural pain, followed after an interval of a few days to two and a half weeks by erythema nodosum. For positive diagnosis, however, either a positive skin test or a positive culture from the sputum or both are required. Typical double contoured spherules, with or without endospores, can sometimes be demonstrated in fresh sputum preparations and provide a valuable clue, but they cannot be accepted as conclusive diagnostic evidence without confirmation by culture and animal inoculation.

The routine skin test consists of an intracutaneous injection of 0.1 c.c. of a 1 : 1.000 solution of coccidioidin (0.1 mg.). The reaction begins to appear in about twelve hours, is well marked at twenty-four hours, and reaches its maximum size and intensity in about forty-eight hours. It is closely similar to the tuberculin reaction, with both erythema and induration and, in some instances, vesiculation. Sputum or gastric contents should be concentrated by centrifugation and the sediment examined microscopically in the wet preparation without staining for coccidioies spherules.

Kessel (1939) investigated the clinical value of the *Coccidioidin skin test.*

Reports of positive skin tests in coccidioidal granuloma infections in both man and animals have been given by a number of observers, among them, David (1924), Jacobson (1932), Hirsch and Benson (1927), Beck, Traum and Harrington (1931), Gifford, Buss and Douds (1937), and Hurwitz, Young and Eddie (1937). However, positive results in all infected cases and negative results in all uninfected animals and cases have not been consistently reported. Most workers are agreed that active cases of coccidioidal granuloma yield positive intradermal tests but the question of greatest uncertainty is with reference to possible cross reactions with tuberculosis.

Beck et al. (1931) first raised this question when they reported weakly positive tuberculin reactions in animals infected with *Coccidioides immitis.* Hurwitz, Young and Eddie (1937) also reported positive coccidioidin reactions in patients, having no known coccidioidal infection. Gifford and associates (1937), however, have reported findings in an extensive survey in Kern County, California and in reactions with "San Joaquin Fever" patients which indicate specificity of the coccidioidal reaction and show a high incidence of positive tests in individuals living in that area.

Summary of coccidioidin skin tests.

	Coccidioidin test		Remarks
	+	—	
26 human cases proved by laboratory methods . .	22	4	Negative results only in terminal stage of disease
228 Tuberculosis patients .	37	191	Majority of reactors give positive occupational and residence history
29 guinea pigs inoculated with *C. immitis*	29		Tuberculin tests negative
27 guinea pigs inoculated with *M. tuberculosis* . .		27	Coccidioidin tests negative Tuberculin tests positive

Active cases of coccidioidal granuloma, prior to the final fatal stages give a positive skin reaction. Tuberculosis patients tested with coccidioidin as a rule give negative reactions. From most of the positive reactions observed in this study it has been possible to obtain a history compatible with exposure to coccidioides infection. Guinea pigs experimentally inoculated with *Mycobacterium tuberculosis* give a negative coccidioidin skin test and a positive tuberculin test. Guinea pigs experimentally inoculated with *Coccidioides immitis* give a positive coccidioidin skin test and react negatively to tuberculin skin tests. These results indicate that the coccidioidin skin test possesses a high degree of specificity and parallels in many respects the tuberculin test in its mode of action. It can be used as an aid to diagnosis but probably no more reliance should be placed on it than is usually given the tuberculin test.

FARNESS and MILLS (1939) reported on *Coccidioides infection* with primary infection in the lung with cavity formation and healing.

Since its recognition in 1892 by Wernicke and Posadas of Buenos Aires, 450 cases with 224 deaths have been recorded to July 1, 1936 in the State of California. With a few exceptions, this represents the cases reported in this country.

The present case is reported because it developed outside of California and because very few clear-cut cases of primary involvement of the lungs are recorded. No cases with cavity formation and subsequent healing by fibrosis are available for study. The very close similarity to acute tuberculosis and blastomycosis and the difficulty in differential diagnosis become evident. This case is still more unusual in view of the extensive pulmonary pathology and the virulence of the invading microorganisms are opposed to the benign clinical course.

Case Report

P. T., a white male, aged 15 years, Michigan born, gave a history of severe nasal sinus disease and bilateral middle-ear infection over a period of years. In 1928 he had measles, mumps, a bilateral mastoidectomy and pneumonia. Since 1934 he had been attending a private school near Tucson because of the difficulty of controlling the head infections at home in Michigan.

On November 20, 1936, an unidentified insect stung the patient's finger. For half an hour following this sting he experienced acute pain and for a week the finger was numb. Sometime between December 10 and December 15 he contracted a "sinus cold." (It seems improbable that there was any direct relation between the sting on the finger and the so called cold. He was at this same time in contact with the soil through such athletic pursuits as football, riding and rifle shooting.) On January 4 he returned to Tucson from a Christmas holiday at home with a severe "cold," an acute sinus flare-up and discharging ears. He was ordered to the school infirmary and treated for his sinus and ear infection. He was feeling well and had no temperature rise but was coughing and raising some sputum. On January 12 he complained of pains in the right chest. On January 14 the chest was examined and no ausculatory changes were noted. On January 16 an X-ray film was made of the chest which revealed a 3 × 4cm cavity in the right infraclavicular region with a patch of heavy infiltration immediately above the cavity. On January 22 a test of concentrated sputum was negative for tubercle bacilli. A second X-ray film made February 8 showed the cavity to be of the same size but the patch of infiltration had disappeared and the wall of the cavity was exceedingly thin. The patient was feeling well and had no cough or sputum. After further rest a third X-ray film was made February 19 which showed the cavity to be larger and a reappearance of the patch of infiltration above the cavity. In addition there was extensive soft infiltration in the region of the right middle lobe and fluid in the lower left chest.

The laboratory findings on February 22 were as follows: hemoglobin, 16 g.; erythrocyte count 5,380,000; leucocytes 10,500 with 56 per cent neutrophiles, 14 per cent lymphocytes, 3 per cent monocytes and 27 per cent eosinophiles. The blood sedimentation rate by the Westergren method was 7 mm. at the end of an hour. A 24-hour sputum collection weighed 20 g. It was thick, mucopurulent and contained some blood. On repeated examination the sputum contained no acid-fast bacilli but did contain some spherules with double contoured capsules filled with endospores suggesting *C. immitis*. On culture the microorganism grew out as a fungus with long mycelia. Of three guinea pigs inoculated, two with the pure culture and one with concentrated sputum, all showed early orchitis and, at autopsy, the typical tubercular lesions and spherules of coccidioides infestion. *C. immitis* was grown in pure culture from the lesions. The intracutaneous tuberculin test was negative to 0.1 mg. OT. The coccidioidin skin test, performed at a later date, was strongly positive.

The patient was in the hospital two weeks on complete bed-rest. None of the drugs advocated for the treatment of coccidioidal granuloma were prescribed. He continued to gain in weight. His sputum decreased to 3 and 4 g. in 24 hours. He felt well and was irked at having to remain in bed. An X-ray film made February 24, after the second refill, showed a 25 per cent collapse of the right lung. The cavity was the same size although altered in shape and containing fluid. There was clearing of the surrounding infiltration as well as a clearing of the soft lesion on the left. On March 1 the X-ray film showed more compression of the right lung but with the cavity held open by an adhesion. There was further clearing of the parenchymal infiltration around the cavity and also more absorption of the soft lesion on the left. At this time, with the diagnosis definitely established, the artificial pneumothorax was discontinued for fear of producing an empyema.

Interesting features were the absence of constitutional symptoms, a high eosinophilia and, heretofore unreported, early cavity formation. Treatment was rest, artificial pneumothorax, and a specific vaccine. Healing was rapid and after more than ten months' interval (when this report was made) the disease appeared cured.

CRONKITE and LACK (1940) carried out experimental infection with *Coccidioides immitis* causing *primary pulmonary coccidioidomycosis*.

It is of interest to review briefly references in the literature to primary inhalation infection with the fungus. The first suggestion as to the respiratory route of infection was made by OPHULS, in 1904, in a discussion of an autopsied case of coccidioidal granuloma. Again in 1905 and later in 1929 he called attention to the probability of infection through the pulmonary route. Other authors who have mentioned the probable importance of inhalation of the spores of the fungus are: MCNEAL and TAYLOR, CUMMINS and SANDERS, HAMMACK and LACEY, RIXFORD, SORSKY and NIXON, BECK, TRAUM, and HARRINGTON, and DE ALMEIDA. DICKSON has repeatedly emphasized the pulmonary portal of entry and has been largely responsible for the undertaking of the experiments reported here. The epidemiological survey of San Joaquin fever by SMITH and his associates adds further clinical proof of the respiratory nature of the primary infection. Besides these pathological, clinical, and epidemiological references, there are a few experimental studies. In 1906 BROWN attempted to infect guinea pigs by transferring cultures in the spore-bearing stage to their nostrils and their food. However, before completion of the experiments his animals were destroyed in the San Francisco fire, and there has been no subsequent report. Jacobson unsuccessfully attempted to transmit the disease to guinea pigs kept in the same cage with or fed the leavings of infected animals. The first successful pulmonary infections were described by AHLFELDT who exposed a small number of rabbits and guinea pigs by tracheal insufflation and by spraying a suspension of the fungus into a closed chamber. She reported that three guinea pigs exposed to the spray died within 11 days showing a weight loss of about 45 per cent. Autopsies were negative in the gross, but organisms were reported in cervical lymph nodes of two of the animals. PULFORD and LARSON stated that they have experiments under way in which spores are placed in tubes which fit over the guinea pigs' mouths. Thus far, their results have not been reported.

STEWART and KIMURA (1940) published on the *skin test for coccidioidal infection.*

In 1915 COOKE investigated the serology of *Coccidioides immitis* (Stiles). He reported the demonstration of precipitins and that there was no evidence for the existence of agglutinins or antitoxins. His results with a skin test were equivocable. One year later, CUMMINS and SAUNDERS presented evidence supporting the specificity of the skin test in the detection of coccidioidal infections. These investigators grew coccidioides in a broth medium for about 37 days and then employed the filtrate of their cultures in the experiments. These filtrates were a composite of seven "strains." A more complete description of the skin test was published by DAVIS in 1924. In 1927 HIRSCH and BENSON, HIRSCH and D'ANDREA published 3 significant papers. The results of this trilogy may be summarized as follows: (a) the investigators used a synthetic medium composed of sodium chloride 0.5 %, ammonium lactate 0.6 % and dibasic potassium phosphate 0.2 %; (b) in this medium, *Coccidioides immitis* liberated a soluble substance which, in animals infected with coccidioides and in patients with coccidioidal granuloma, caused a skin reaction analogous to the tuberculin reaction they also described an immediate reaction similar to the wheal produced by pollen extracts in allergic skin tests; (c) this soluble substance was not destroyed by heating to 80 C. for 30 minutes; prolonged electrodialysis of a filtrate resulted in the separation of white floccules soluble in N/100 sodium hydroxide and solutions of this substance caused a skin reaction like those of ordinary filtrates; (d) the active substance was precipitated by alcohol, soluble in 0.85 % sodium chloride, dilute acids and alkalis, it contained 3 to 4 % nitrogen and the osazone prepared from an hydrolyzed solution resembled dextrosone in structure; (e) guinea pigs could be sensitized with the broth culture filtrates and with the precipitates of the filtrates as well as with the dried mycelium. Furthermore, there is no standard procedure for the preparation of coccidioidin, no method for evaluating the potency of the various preparations and no standard dose for testing patients suspected of having coccidioidal infection. Preparation of Coccidioidin. — The culture solution selected was the synthetic medium employed in the metabolism studies of *Coccidioides immitis* and *Blastomyces dermatitidis.* This medium was most likely to eliminate nonspecific skin reaction that might be provoked by proteins or ingredients incorporated in the usual types of culture solutions. The composition of the medium is:

Ammonium chloride	1.0	gm.
Sodium acetate	1.0	gm.
Dibasic Potassium phosphate	0.2	gm.
Monobasic Potassium phosphate	0.2	gm.
Magnesium sulphate	0.01	gm.
Distilled water	100	cc.

The culture solution was distributed into 2 liter Erlenmeyer flasks in 1,000 cc. amounts and then autoclaved at 15 pound pressure for 30 minutes. The medium was then inoculated with a culture of *Coccidioides immitis* (Stiles) grown on beef extract agar slants. After inoculation the cultures were incubated at 37 C. together with uninoculated control flasks which also contained 1,000 cc. of sterilized culture solution. The clear colorless medium became more and more tinted until at the end of the ninth month it was pale amber in color. This was in distinct contrast to the control flasks which remained colorless. After 12 months the contents of the inoculated flasks were triturated for 24 hours in the ball mill described by KRUEGER. The resulting mixture was then filtered through 2 % collodion filters. Total nitrogen and non protein nitrogen determations were made on the filtrates using micro-Kjeldahl methods. The filtrates were lyophiled according to the method described by FLOSDORF and MUDD. The problem is to define "one skin unit." The unit must refer to a definite quantity of reactive material and this quantity should, in sensitized subjects, provoke reactions that are easily read and interpreted. These reactions ought to occur within a given period of time and persist for a definite interval. Further, this amount of reactive substance must be contained in a volume of material ordinarily employed for intradermal tests. This is usually 0.1 cc. Arbitrarily then, one skin unit is defined as follows: One skin unit is the smallest amount of reactive substance contained in 0.1 cc. of solution that will on the average, in sensitized subjects, provoke in 24 hours an erythema of 1.0 cm. in diameter persisting for 24 hours. The exact chemical nature of coccidioidin is not known but HIRSCH and D'ANDREA have shown that the active substance contains nitrogen associated with a carbohydrate. The non-protein nitrogen fraction is chosen in these studies because to date the experimental work does not substantiate the concept that the protein fraction is involved in the skin test. The results of HIRSCH and his associates suggest that the active preparation of coccidioidin is a carbohydrate.

C. E. SMITH (1940) carried out substantial investigations on *epidemiology* of *acute coccidioidomycosis* with *erythema nodosum* ("San Joaquin" or "Valley Fever").

1. An investigation was made of 432 patients with "San Joaquin fever," "valley fever," or "desert rheumatism" in Kern and Tulare Counties during the 17 months beginning December, 1937. All recovered without sequelae. 2. This disease, characterized by influenza-like prodromes followed by erythema nodosum with or without erythema multiforme, was frequently confused with such communicable diseases as influenza, pneumonia, tuberculosis, measles, and smallpox, and occasionally even with poliomyelitis, typhoid fever, and syphilis. 3. The incubation period ranged between 1 and 3 weeks, probably most frequently requiring 2 weeks. 4. Sensitivity to coccidioidin, a product of the causative fungus, *Coccidioides immitis*, was established in from 2 to 17 days after onset of symptoms, generally in the second week of the illness. 5. The erythema nodosum was associated with the hypersensitivity of freshly acquired allergy. This allergy, apparently like tuberculin sensitivity, was of long duration. Consequently, second attacks of "San Joaquin fever" were very rare (not more than 2 in this series). 6. Coccidioidomycosis with erythema nodosum was rarely if ever contact infection. The endosporulating spherules of *Coccidioides* occurring in man and animals did not pass directly from host to host. Apparently, the disease was acquired by inhalation of the chlamydospores. 7. The seasonal incidence corresponded to the climate and the agricultural activities, with the peak in the dusty fall and the ebb in the wet winter. 8. The benign "valley fever" was most common among white females in contrast with coccidioidal granuloma which is known to be especially prevalent among dark-skinned males. 9. Nearly half the patients had resided in the San Joaquin Valley less than 1 year, while only one-ninth had been in the region

10 years or more. The predilection for new comers corresponded with the distribution by length of residence of coccidioidin sensitivity. 10. Eventually most of the inhabitants of the region undergo an infection with *Coccidioides*. As not more than 5 per cent of those infected develop erythema nodosum, this series represented between 8,000 and 10,000 attacks of coccidioidomycosis.

TABLE I. *Time Intervals between Clinical Onset, Erythema Nodosum Coccidioidin Reactions in Certain Cases of Acute Coccidioidomycosis*

Patient	Period in Days from Onset Until		
	Negative Coccidioidin	Erythema Nodosum	Positive Coccidioidin
J.C.B.	2	17	19
O.G.	3	11	12
J.A.J.	8	15	20
A.S.	10	18	20
J.W.	17	21	40
J.D.	—	3	3
T.Y. ¹)	—	6	4
B.B. ¹)	—	10	8
A.C. ¹)	—	12	6
M.M. ¹)	4,9	13	11
R.R. ²)	11	—	14
E.B. ²)	1 (or 2)	—	2 (or 3)

TABLE II. *Seasonal Distribution of San Joaquin Valley Fever Onsets Kern and Tulare Counties*

Year Month	First Half	Second Half	Total	Year Month	First Half	Second Half	Total
November, 1937	—	9	9	August, 1938	22	30	52
December, 1937	10	10	20	September, 1938	18	23	41
January, 1938	5	5	10	October, 1938	35	30	65
February, 1938	7	7	14	November, 1938	20	23	43
March, 1938	6	2	8	December, 1938	24	10	34
April, 1938	3	6	9	January, 1939	6	5	11
May, 1938	4	7	11	February, 1939	6	5	11
June, 1938	8	13	21	March, 1939	10	16	26
July, 1938	20	16	36	April, 1939	5	5	10

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¹) Note positive coccidioidin prior to erythema nodosum.

²) Never developed erythema nodosum.

TABLE III. *Age Distribution of San Joaquin Valley Fever Patients Kern and Tulare Counties November,* 1937 *to May,* 1939

Age	Number	Percentage of total
0 — 9	45	10.4
10 — 19	110	25.5
20 — 29	119	27.5
30 — 39	76	17.6
40 — 49	49	11.3
50 — 59	24	5.6
60 — 69	9	2.1
Total	432	100.0

TABLE IV. *Sex Distribution of Valley Fever Patients by Age and Ratio of Males to* 100 *Females Kern and Tulare Counties,* 1937—1939

Age	Number		Males per 100 Females
	Males	Females	
0 — 14	44	57	77
15 — 24	31	70	44
25 — 34	28	80	35
35 — 44	15	53	28
45 — 54	9	28	31
55 — 64	4	10	40
65 plus	1	2	50
Total	132	300	44

Table 4 shows that one-third of the male cases were under 15 and that thereafter the proportion diminished steadily. Howerve, only one-fifth of the females were in the school group and the proportion increased for two decades before diminishing. The predilection for females is noteworthy. It is in line with the experience in tuberculous erythema nodosum. Under the age of 15, the ratio of 8 males to 10 females was not striking. However, from puberty to menopause the females greatly outnumbered the males. In the fifth decade there were only 2 males per 10 females although here the numbers were small. For the entire group there were only 4 males to 10 females. On the other hand in coccidioidal granuloma this ratio is reversed: 40 to 70 males per 10 females.

Table V. *Length of Residence in San Joaquin Valley of Kern and Tulare County Patients seen from December,* 1937, *to May,* 1939 *Total Number of Cases* 432

Per cent of total	
Under 6 months	26.2
6 — 12 months	17.8
1 year	19.4
2 — 4 years	19.4
5 — 9 years	6.1
10 years and over	11.1
Total	100.0

Cumulative Percentage	
Under 1 year	44.0
Under 2 years	63.4
Under 5 years	82.8
Under 10 years	88.9
All	100.0

The birth states of the patients (Table 6) confirm these figures. There were only two-thirds as many "Native Sons and Daughters" as "Oakies". One-half the patients had been born in Oklahoma, Arkansas, and Texas.

Table VI. *Birth States of Kern and Tulare County Valley Fever Patients Seen From December,* 1937 *to May,* 1939 *Total Number of Cases* 432

State	Per cent of total	
California	18.1	
Oklahoma	27.5 —	51.8
Arkansas	12.5 —	
Texas	11.8 —	
Other Southern States	8.1	
Other States and Foreign	22.0	
Total	100.0	

Foley et al. (1940) reported on a case of *Coccidioidal granuloma originating in Texas.*

A man, an office worker, aged 33 years, registered at the clinic January 25, 1937. He complained of fever which occurred afternoons and evenings ranging from 99 to 101 F. (37.2 to 38.3 C.), chronic productive cough, night sweats, chronic fatigue, headache, gaseous indigestion, transient blurring of vision and a chronically draining sinus posterior to the right ear. The patient always had been well until July, 1927, when he was suspected of having typhoid fever because of a generalized feeling of malaise and a slightly elevated temperature. Three days after the injection of serum for the relief of this condition, chills developed, temperature increased and he experienced nausea. These symptoms persisted intermittently for six weeks. During this period a chronic, hacking, nonproductive cough developed and the man lost 43 lb. (19.5 kg.). He regained his lost weight and strength slowly and remained fairly well until August, 1933, when what appeared to be a subacute attack of appendicitis occurred. The pain in the right lower quadrant of the abdomen subsided in a few days but returned in February, 1934, and a slightly inflamed appendix was removed. Convalescense was prolonged for ten days by an unexplained fever of slight degree which presisted after the wound was completely healed. In March 1934, a dull aching pain developed in the upper part of the right maxilla in the region of the first molar tooth. This distress continued intermittently in spite of the removal of several upper and lower molar teeth on the right side, drainage of the right antrum and tonsillectomy. With the onset of headaches in the right parietal and occipital regions and transient blurring of vision of the right eye, the possibility of a tumor or abscess of the brain was considered. In July, 1935, the patient noticed two small, tender, swollen regions each about the size of a pea over the right occipital region. These were aspirated in October, 1936. The attending physician reported that spore-like bodies were present in fresh smears of the aspirated material. Cultures of the aspirated material and examination of the stools both revealed a fungus-like growth and a diagnosis of sporothrix infection was made. Roentgenologic examination of the skull performed elsewhere in October, 1936, revealed two bony defects each about the size of a pea in the right occipital region. Because of these findings open drainage and irrigation of the infected region with a solution of iodine had been instituted. A milky fluid continued to drain from a small sinus in the right occipital region. The patient was advised to take from 100 to 180 drops of a saturated solution of potassium iodide daily for three months. He noticed only temporary improvement and finally decided to consult The Mayo Clinic in January, 1937. The past medical history was irrelevant except for a previous roentgenologic diagnosis in 1933 of bilateral pulmonary tuberculosis. The patient never had lived in California. Roentgenologic examination of the head revealed three irregular, rounded regions of destruction in the lambdoidal suture and in the occipital bone. There was some thickening of the bone in the surrounding region consistent with old residual effects of osteomyelitis. Lesions of both upper lobes of the lungs, simulating active tuberculosis were revealed on roentgenologic examination of the thorax. Tuberculosis, syphilis, sporothrix infection and the possibility of growth of a fungus as a secondary invader of the usual type of chronic osteomyelitis that involves bones as a chronic process were considered in the differential diagnosis. Finally, the possibility that the lesion of the skull resulted from infection by *Coccidioides immitis* which began in the lungs and later involved the skull owing to metastasis and possibly also involved the gastrointestinal tract was considered. Microscopic examination of the tissues removed at operation revealed the presence of *Coccidioides immitis*. Operation February 5, 1937, under intravenous anesthesia (because of the pulmonary lesion) with pentothal sodium, a curved incision was made over the right occipital region and the fluctuant mass and region of osseous destruction were exposed. A mass of shiny, silvery, granular tissue was exposed. This had an appearance not unlike that of a pearly, intradiploic, epidermoid tumor. However, a capsule could not be demonstrated. The same type of material exuded through all three defects of the skull. These openings, except for the lower mesial one, had extended entirely through the bone of the skull. The three holes were joined by rongeuring away the bridge of bone between them and by this procedure an extensive granuloma was uncovered. Specimens were taken for cultures and for smears. The lesion was intimately adherent to the dura mater. Sufficient bone was removed to get beyond the diseased portion and to uncover normal dura mater. The granulomatous mass was scraped carefully from the meninges. The wound was swabbed with tincture of iodine and a long strip of gauze which had been soaked in tincture of iodine and had been wrung out was placed between the dura mater and the scalp to fill the dead space and to control oozing from the raw bony edges. The wound was closed with interrupted sutures. Postoperative course — the patient experienced very little reaction after the operation. The temperature remained normal after the removal of the gauze packing on the fifth postoperative day. The patient was dismissed from the hospital on the eleventh postoperative day and was released from the care two days later at which time his wound was healed and his condition was satisfactory. The patient was given 15 gr. of thymol (1.0 gm.) three times a day and was advised to take the same amount six times daily unless untoward symptoms occurred. He continued the use of thymol and received a diet high in caloric content and in vitamins. About two weeks after his dismissal from the clinic, a course of Dr. H. P. JACOBSON'S vaccine was given. Subsequent letters from the patient (last letter, September 26, 1938) revealed that, for seven months before returning to work, the patient followed a strict regimen similar to that advised for patients who have tuberculosis.

KESSEL (1941) presented his recent observations on *Coccidiodes immitis.* Between the years 1893 and 1939, 660 cases of coccidioides have been reported to the California State Department of Public Health. Chart 1 shows the occurrence of these by years. The actual geographical origin of the infections is not known in all cases but the majority of the reported cases originate in the southern portion of the San Joaquin Valley. Of 100 cases of coccidioidal granuloma observed in the Los Angeles County Hospital in recent years, 51 probably originated in the San Joaquin Valley, 31 in the counties south of Tehachapi in Southern California and 8 in Arizona, New Mexico or Texas (chart 2).

CHART I. *Coccidioidal granuloma in California*

1893 — 1899	4
1900 — 1904	7
1905 — 1909	9
1910 — 1914	17
1915 — 1919	23
1920 — 1924	50
1925 — 1929	123
1930 — 1934	163
1935 — 1939	264
Total	660

CHART II. *Coccicioical granuloma cases in Los Angeles County Hospital*

Ages	No.	Race	Sex	Probable Orgin of Infection
1—10	9	Caucasian 32	Male 84	San Joaquin Valley 51
11—20	11	Mexican and Oriental 51	Female 16	Southern California 31
21—30	34	Negro 17		Arizona, New Mexico, Texas 8
31—40	24			Not determined 10
41—	22			

The coccidioidin has always been prepared from a mixture of 5 strains of the fungus isolated early in the work, while STEWART working with antigen prepared from single strains did not procure clear cut results. Recently the five

CHART III. *Distribution of lesions in cases of Coccidioidal Granuloma*

Initial Lesion	Pulmonary	Active Lesions at Illness or Death	
		Cutaneous	Meningitis without lung
Lung 29	Lung only 8	Cutaneous only 4	Meninges and bone 1
Skin 13	Lung and Cutaneous 15	Cutaneous and Bone 3	Meninges only 1
Uncertain 8	Lung and Generalized 16		Meninges and Generalized 2

strains of coccidioides used in preparation of antigen in this laboratory have been inoculated into guinea pigs, 4 animals being inoculated with each strain. All animals were then given coccidioidin skin tests with the respective homologous and heterologous antigens, prepared from each strain of fungus. Most reactions were positive, with both homologous and heterologous antigens. The few negative reactions could be explained by the fact that the stage to which the infection had progressed when the test was given was either too early or too late in the course of the disease for one to expect a positive skin test. Recovered cases of coccidioides have been known to give a positive coccidioidin reaction for as long as 9 years after the initial infection and one may assume that individuals who give a positive coccidioidin test have experienced a previous infection. Evidence in favor of this possibility has been presented by Cox and SMITH who have demonstrated arrested pulmonary coccidioidal lesions in autopsies. Dr. E. M. BUTT of this laboratory, by personal communication reports a series of 8 individuals critically ill with some disease other than coccidioides who were given the coccidioidin skin test and at subsequent autopsy, were searched carefully for arrested coccidioides lesions. Six of the cases tested gave negative reactions and 2 were positive. The negative reactors exhibited no coccidioides lesions. One postive reactor died of primary carcinoma of the liver and exhibited healed pulmonary lesions from which a postive culture of *Coccidioides immitis* was obtained. In the other, who died of a cardiac disease, it was possible to demonstrate the organisms in the lung tissue though no growth of the fungus was obtained. Such observations provide additional support to the specificity of the coccidioidin skin test.

THORNER (1941) investigated in *Coccidioidomycosis* the relative values of *Coccidiodin* and *Tuberculin* testing among *children* of the San Joaquin Valley.

1. Two hundred sixty-seven children, taken at random from the Pediatric Clinic of the Kern General Hospital, and ranging from the ages of 0—19 years, were tested simultaneously with 0.1 cubic centimeter of Koch's O.T. 1 : 1.000 (0.1 milligram) and coccidioidin 1 : 100 (1 milligram).
2. It was found that there was a noticeable higher incidence of coccidioidin over tuberculin reactors as the length of residence in the county increased.
3. The low ratio of "valley fever": coccidioidin reactors noted in this and other surveys points to the relatively benign as well as universal nature of the primary phase of coccidioidomycosis infection as it exists in the San Joaquin Valley.
4. Progression into the later and severe granulomatous stage of the disease only occurs when tissue and lymphatic resistance are overcome and generalized dissemination takes place via the blood stream.

TABLE I. *Age Groups*

Two hundred and sixty-seven children, ranging in age from five months to nineteen years, were included, and divided into four groups

Group	No.	Ages	Per cent Positive to	
			Coccidioidin	Tuberculin
I	43	0—4 yrs.	31 %	30 %
II	108	5—9 yrs.	52 %	36 %
III	106	10—14 yrs.	61 %	42 %
IV	10	15—19 yrs.	60 %	40 %
Total . . .	267			

TABLE II. *Sex Distribution*

Sex	No.	Per cent Positive to	
		Coccidioidin	Tuberculin
Malet.	116	54 %	38 %
Female	151	54 %	38 %
Total	267		

TABLE III. *Race Distribution*

Race	No.	Per cent Positive to	
		Coccidioidin	Tuberculin
		No.	No.
White.	216	51 % (111)	31 % (67)
Mexican	35	54 % (19)	65 % (23)
Negro	13	61 % (8)	61 % (8)
Indian	2	(2)	50 % (1)
Chinese	1	100 % (1)	(1)

TABLE IV. *Length of Residence in Kern County*

Years in County	No.	Per cent Positive to	
		Coccidioidin	Tuberculin
Less than one year . .	40	25 %	32 %
1—4 years	108	49 %	33 %
5—9 years	69	70 %	41 %
Ten years and over . .	50	76 %	25 %
Total	267		

FARNESS (1941) reported on *Coccidioidomycosis.*

The coccidioides fungus is known to infect cattle, sheep and dogs as well as human beings. No age is immune to the infection. The portal of entry, except in the occasional primary cutaneous lesion due to trauma, is the respiratory passages. The dissemination of the infection occurs through the blood and lymph streams and by direct extension from the primary focus. The disease may involve any organ in the body, but the lungs, skin, bones, lymph nodes and meninges are most commonly affected. The incubation period is still in doubt, although cases in which the probable time of infection is known would indicate that it is between one and three weeks. Conditions suitable for maintaining the infectivity of the the organism are found in places in which there is some rain and little cold, permitting easy distribution of the chlamydospores with the dry soil of the region. When one realizes that many areas of the dry Southwest meet these requirements, it becomes apparent that the possibilities for the maintenance and spread of the disease are considerable. Coccidioidal disease is protean in its clinical manifestations, and its mimicry of tuberculosis and blastomycosis is such that a diagnosis can be made with certainty only by finding the characteristic spherules in the tissues, pus or sputum and confirming this discovery by culture and guinea pig inoculation. A good routine diagnostic method is to concentrate sputum, gastric contents, or other likely material, from a suspected patient and examine the sediment in a wet preparation without staining for the typical coccidioides spherules. Part of the sediment is then cultured on SABOURAUD'S medium and part injected intraperitoneally and intratesticularly into guinea pigs. On culture, a white fluffy growth is obtained which is composed of mycelium and chlamydospores. In the inoculated guinea pigs, abscess formation and recovery of the diagnostic spherule is accomplished in ten to thirty days. A specific cutaneous reaction is obtained in infected patients. Coccidioidin, as prepared by Dr. CHARLES E. SMITH of Stanford University School of Medicine, has been widely used. The technic is simple and similar to that used in tuberculin testing. One tenth cc of a 1 : 1,000 solution is injected intradermally and the reaction determined in forty-eight hours. A positive reaction, like the tuberculin reaction, is accompanied with erythema and induration and sometimes vesiculation. If the 1 : 1,000 dilution test gives negative results, a 1 : 10 dilution is used. Syringes previously used for tuberculin testing must not be used for coccidioidin testing because false positive reactions are apt to occur. From observations and available data, it would appear that the reaction is specific, develops early in the course of the disease and persists indefinitely. In this community, several groups have been given cutaneous tests with interesting results. Out of 43 patients in the Southern Pacific Tuberculosis Sanatorium most of whom were not residents of Arizona, 10 per cent reacted positively. Among 60 patients at the Pima County General Hospital who were citizens of Arizona, there were between 20 and 30 per cent positive reactors. Of 141 Pima Indian School children given cutaneous tests by Dr. J. D. ARONSON, of the Henry Phipps Institute of Philadelphia, 90 per cent gave positive reactions. These results definitely establish the presence of a focus in the Tucson area. In the accompanying table are listed the results of coccidioidin cutaneous tests from various sections of the country.

Evidence is accumulating that foci for coccidioidal infection exist in areas outside California. Five cases of the disease occurred in Arizona and were apparently contracted in this locality. Of these 5, 3 were fatal, 2 during the acute stage of the infection and 1 in the chronic granulomatous stage.

Coccidioidin Cutaneous Tests in Endemic and Nonendemic Areas of the United States

Place where Study was made	No. of Patients	Number Positive	Percentage Positive
A. In Patients with Coccidioidomycosis			
San Francisco	12	12	100
Los Angeles	26 1)	22	84.6
B. In Tuberculous Patients (Cross Reaction?)			
Michigan	425	143	Controlled series; no history of residence in endemic areas
Tucson	73	16	21.9 — Endemic areas
Los Angeles	228	37	16.2 —
San Francisco	177	49	27.5 — No data as to residence
Oakland, Calif.	267	46	17.2 —
Philadelphia	117	7	5.9 —
New York	300	0	0.0 —

C. In Nontuberculous subjects in Areas Endemic for Coccidioides Immitis

Place Where Study was Made	Type of Person	Number of Persons	Number Positive	Percentage Positive
Kern County, Calif. (San Joaquin Valley)	Workers in road camp	60	15	25
Kern County, Calif. (San Joaquin Valley)	School children	3,307	1,924	58.2
San Joaquin Valley, Calif.	Patients in Hospital	184	37	21
Pima Indian Reservation, Gila Crossing Ariz.	School Children	141	127	90.1
Tucson	Patients in medical service	42	7	16.9

D. In Nontuberculous Subjects in Areas Not Endemic for Coccidioides Immitis

Place Where Study was Made	Type of Person	Number of Persons	Number Positive	Percentage Positive
Ann Arbor, Mich.	University of Michigan Students	70	0	0.0
Ann Arbor, Mich.	Patients in Medical Service	76	1	1.31
San Francisco	Patients in Medical Service	260	11	4.23
Philadelphia	School Children	15	0	0.0

GRAIG and DOCKERTY (1941) gave a brief review with report of a case of *meningeal involvement* in *Coccidioidal granuloma.*

A short account is given of the history of *Coccidioides* and the geographic distribution of the disease which it causes. In the case of coccidioidal granuloma the symptoms were the result of intracranial involvement, but the primary lesion was of pulmonary origin. The infection was almost certainly acquired by the patient during a brief sojourn in southern California.

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1) Four patients were in terminal stage.

TABEL I. *Tissue Diagnosis: Differential Points*

Organism	Disease	Reaction	Cellular Products	Budding	Spores	Distribution of Lesions
Blastomyces dermatitidis	Blastomycosis	Acute, with polymorphonuclear neutraphils	None	Always	Never	Skin and generalized (lungs especially)
Coccidioides Immitis	Coccidioidal granuloma	Acute, with polymorphonuclear neutrophils	None	Never	Always	Skin and generalized (lungs especially)
Debaryomyces neoformans	Torulosis	Mild chronic	Mucoid Material	Sometimes	Never	Usually in brain, meninges

TABLE II. *Laboratory Diagnosis: Differential Points*

Organism	Disease	Organisms in culture			Pathogenicity for animals
		Mycelia	Ascospores	Colonies	
Blastomyces [1] *dermatitidis*	Blastomycosis	Always	Never (?)	Dry and fuzzy	Slight or absent
Coccidioides immitis	Coccidioidal granuloma	Always	Never	Dry and fuzzy	Marked for all laboratory animals
Debaryomyces neoformans	Torulosis	Never	Recently demonstrated	Moist (mucoid)	Marked for rats and mice; slight for guinea pigs (principally brain lesions)

POWERS and STARKS (1941) reported on *roentgen findings* in a group "epidemic" of *acute primary Coccidioidomycosis*.

This report deal specifically with a group of college students who presumably contracted the disease at the same time and place. During a three-day trip to an endemic area, 7 of the 14 students were subjected to particularly dusty conditions and all 7 contracted the disease. All eventually became positive to coccidioidin tests and all showed positive X-ray findings in varying degrees. There are no reference to the primary form of coccidioidomycosis in the roentgen literature, though roentgen findings have been described in the general literature by FABER, SMITH, and DICKSON. Fuzzy densities were seen at the lung roots extending for variable distances into the lung fields, in some instances out to the periphery and having the appearance of consolidation with lobar distribution. FARNESS gives a similar description, stating that "X-rays show hilar thickening and dense shadows scattered through the pulmonary fields." CARTER states that miliary dissemination is probably the rule. (CARTER'S report deals with the chronic form.) The most complete report on the roentgen findings in coccidioidal granuloma is that of CARTER, dealing with the chronic or granulomatous form. His findings were similar to those already mentioned, with additional emphasis on the frequency of cavitation and pleurisy. In 2 of 7 patients, roentgenograms were made on the third day from the onset of symptoms; in 4 between the seventh and fourteenth day; in 1 on the twenty-first day. All 7 cases gave positive X-ray findings, but these varied considerably as to the extent of the involvement. These cases suggest two types of primary reaction: one a more severe infection with multiple lesions from the start; the second a milder type with solitary or few lesions, running a more benign and shorter course. The severe type with multiple lesions may be progressive, with new lesions appearing over several weeks, some going on to cavity formation. Cavities seem to form and close fairly rapidly. Many of the lesions show central transparency at about three to four weeks, suggesting necrosis or caseation. The one case of this type has one small cavity remaining after one year. Another cavity closed after a few weeks. Several other lesions have remained as homogeneous nodules, simulating ASSMANN'S foci of tuberculosis. The majority of cases show solitary or a few fairly sharply contoured spherical, opaque areas having a peripheral distribution. The location is usually opposite or posterior to the hilum, in the apex of the lower lobe, or the lower part of the upper lobe. Apices appear to be spared but lesions may be present at the bases, and pleural "tenting" at the base was present in one case. The lesions are usually homogeneous in density and many of them appear distinctly nodular. In the majority of the cases some shrinking is noticeable in about one week. Some lesions remain as solitary homogeneous nodules about one-fourth the size of the original focus. Others are absorbed, leaving an inconspiciuous parenchymal scar. No calcification can be demonstrated in any of these nodules as yet (one year). Increase in hilar density has not been a prominent feature in any of these cases. Lateral examination usually shows the opacities to be peripheral and posterior to the hilum. It is doubtful if acute coccidioidomycosis can be diagnosed by X-ray alone, but the roentgenograms may be suggestive enough to point the way to a correct diagnosis. The multiple lesion type may simulate metastatic disease or scattered embolic foci of infection (beginning abscesses), but the clinical picture, eosinophilia, finding of spherules in the sputum, and (later) agglutination reaction and the coccidioidin test will settle the diagnosis. The milder case (those with solitary or few lesions) may simulate pneumonia, but bronchopneumonia usually shows more "patchy density" and less homogeneity. Areas of opacity in lobar and pseudolobar pneumonias are usually larger and not so spherical. An "epituberculosis" reaction is often the first diagnosis, but primary coccidioides lesions fail to show the fading off into the surrounding lung fields, are more spherical, lack the marked hilar reaction, and usually clear more rapidly. Symptoms are more severe in coccidioides. Some lesions may simulate a cyst or tumor but the history, clinical picture, and laboratory findings should make differentiation possible. Solitary lesions may simulate pneumonic infiltrations, but all appear to have little in common with the "raisin-on-the-stem" appearance of basal bronchopneumonia. They usually are and remain more circumscribed than an early pneumonic patch. Lesions posterior to the hilum are more likely to be confused with a beginning pneumonia or with the early stages of lung abscess. FRIEDLANDERS bacillus pneumonia may be simulated by severe cases with multiple foci. We have no information as to the roentgen appearance in other primary fungus infections. The subject of the roentgenologic aspects of bronchomycosis has been throughly presented by DOUB, whose cases included blastomycosis, actinomycosis, moniliasis, aspergillosis, coccidioidal granuloma, and streptothricosis. Most of these cases were of several months' duration and, with the possible exception of blastomycosis, appear to show little similarity to acute coccidioidomycosis. This does not apply to the granulomatous form, however, as the evidence indicates that its appearance may often be quite similar to that of several of the bronchomycoses.

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[1] The fungus "*Zymonema dermatitidis*" has been named by some as the etiologic agent in blastomycosis. However, because absolute proof of this is lacking, HENRICI and others are in favor of retaining this older term, manifestly inaccurate, but justified on the basis of usage.

[2] See footnote of Table 1.

WINN and JOHNSON (1942) made a *roentgenographic study* of 40 cases with *primary Coccidioidomycosis.*

They have observed 40 cases of primary coccidioidomycosis throughout the period of clinical illness and until the roentgenographic pulmonary features have either cleared, remained stable, or progressed. Admittedly, it will be necessary to repeat these observations over many more cases before we can finally evolve a clear-cut understanding of the pathogenesis of the infection as revealed by serial roentgenographic observations. Recognizing the fact that the initial infection is for all practical purposes a pulmonary one (CARTER), only the primary manifestations as disclosed by roentgenography of the lungs will be considered.

From the series of cases they have selected certain ones as illustrative of important roentgenographic aspects of the primary coccidioidal infection. It was possible in most instances to isolate *Coccidioides immitis* from the sputum. Positive cutaneous reactions to coccidioidin were also present in each case. Serologic studies, in the majority of the patients, revealed the presence of circulating antibodies, and not only confirmed the diagnosis but were suggestive of the particular phase of the infection.

Depending upon the amount of infection, acute primary coccidioidomycosis varies considerable in the degree of pulmonary involvement. A small nodular area of opacity may be all that is visible and on the other hand there may be single or confluent areas of pneumonitis, more usual in the pulmonary bases. In many instances the lesions appear to be predominantly exudative in character and clear fairly rapidly. Occasionally, they may leave a small amount of residual fibrosis. In the Negro and Filipino, perhaps owing to racial susceptibility, they have observed such primary exudative lesions to progress and result in early fatal dissemination of the disease. There is a tendency for pulmonary foci of primary coccidioidal infection to assume the appearance of productive lesions, and then clear and decrease in size slowly and incompletely, leaving nodular densities that suggest caseation and that eventually may undergo calcification. Solitary caseo-fibrotic foci undergoing calcification are illustrated in figure 4 c and d. Figure 4 e is illustrative of a large group of healthy children with calcified pulmonary lesions, who have positive cutaneous reactions to coccidioidin (0.1 cc of 1 : 1,000 dilution) and negative reaction to both old tuberculin (down to and including 0.1 c.c. of 1 : 100 dilution) and to purified protein derivative (second strength). Here, as in primary tuberculous infection, calcification represents a focus of previous coccidioidal infection.

Cavity formation accompanying the early acute phase has been described (FARNESS and MILLS, POWERS and STARKS, and WINN). Such cavities, usually single, may close spontaneously. There is, however, a marked tendency for them to persist, despite regression of the other pulmonary lesions. They then assume a characteristic thin-walled and cyst-like appearance with little or no surrounding collateral reaction, and change little in size, shape, or appearance over a period of several years. Such cavitation may also be multiple. Although these cavities serve as reservoirs for the existence and growth of *Coccidioides immitis,* manifested by the continued presence of endosporulating spherules in the sputum, they apparently cause no injury to the health of the person. They are frequently the source of small repeated hemoptyses which may be the only factor leading to their detection.

Localized bronchiectasis may also result from coccidioidal infection, and in one patient an indolent and benign bronchiectatic process was discovered in the right apex, which progressed very slowly over a period of 11 years. Associated clinical manifestations were slight, consisting only of rare blood-streaked and scanty sputum, and the patient remained in good health.

Initial infection is sometimes manifested by a primary pleuritic effusion, no different from that seen in latent tuberculosis, with the exception that *Coccidioides immitis* may be recovered from the fluid. As observed in one instance, complete absorption of the fluid occurred, with reexpansion of the underlying lung and no residual evidence of the infection other than slight pleural thickening.

Roentgenographic evidence of secondary mediastinal or hilar adenopathy in association with primary coccidioidal infection is infrequent, although it may occur. Such visible adenopathy has, in their experience, proved a sign of ill omen, preceding fatal dissemination of the disease.

DAVIS, R. T. SMITH and C. E. SMITH (1942) reported on an *epidemic of Coccidioidal infection* (*Coccidioidomycosis*).

Endemic regions are, like the site of this small epidemic, semiarid with hot, dry and dusty summers and autumns. The most publicized is the San Joaquin Valley, the southern component of the great central valley, of California. Other parts of southern California, Arizona, Texas and probably New Mexico and Mexico are endemic centers. Sporadic cases have been reported elsewhere in North America and Europe. The only other known endemic area is the dry Chaco region of South America. Many investigators have associated these dusty, arid qualities with the theory that the infection is generally acquired by the inhalation of *Coccidioides chlamydospores*. These spores, which develop in the mycelial form of the fungus, are extremely infectious. The fact that coccidioidomycosis does not pass directly from host to host indicates that the endosporulating spherules, characteristic of *Coccidioides* in man and animals, are not well adapted to disseminating the infection.

These deductions explain other features of the epidemiology of acute coccidioidomycosis such as the maximal incidence in the dusty season, the rapidity with which the infection is acquired and the ultimate infection of more than three quarters of the long time residents. The previous recovery of *Coccidioides* from one set of samples of San Joaquin Valley soil is convincing corroborative evidence. The epidemic described here summarizes this entire epidemiologic concept.

The protean symptomatology of coccidioidal infection is also illustrated in this outbreak. We now recognize that the symptoms of an initial infection are variable. Cases are usually diagnosed "flu" or "bronchitis," although many rarer diagnoses are also made. In from 2 to 5 per cent of such patients erythema nodosum and/or erythema multiforme develops, a symptom complex known colloquially as "San Joaquin fever," "valley fever," "desert fever" or "desert rheumatism."

This first reported epidemic of primary coccidioidomycosis involved 7 out of 14 Stanford University students and faculty members. On the afternoon of April 26, 1940 this group of 10 graduate and undergraduate students together with 4 faculty members left the Stanford campus on a biology field trip to San Benito County, California.

San Benito County is 100 miles southeast of San Francisco astride the Coast Range Mountains. While many parts of the county are well watered and produce diversified crops, those portions on the eastern slopes are quite dry. The Panoche Valley, separated from the San Joaquin Valley only by a low range of hills, averages but 8 inches of rain a year. In the spring the region is suitable for grain raising and grazing, though in the hot parched summers and autumns the country is sere.

The first night the party camped near the highway south of Hollister, the county seat. The following day was devoted to scouring the hills and valleys and collecting a variety of specimens: insects, arthropods, reptiles, flowers and plants. The night of April 27 was also spent in the open in the San Carlos Mountains, part of the Coast Range. On the following day the group returned to Stanford University.

After the diagnosis of active coccidioidal infection was established, blood was drawn for sedimentation and precipitin tests from the other members of the expedition who had symptoms. Specimens of sputum were collected from all who could furnish any. Coccidioidin tests were repeated on most of them.

On the basis of the positive coccidioidin reactions, positive precipitin tests, rapid sedimentation rates, characteristic blood counts and densities in their roentgenograms, all 6 students who had been ill were judged to have had primary coccidioidal infections. Four of the group had *Coccidioides immitis* recovered from their sputums. One other student, J. B., felt well but had a positive reaction to 0.1 mg. of coccidioidin, a positive precipitin test, accelerated sedimentation rate and eosinophilia, bringing the total to 7 infections. The remaining 3 students had been well, gave negative coccidioidin reactions, had normal sedimentation rates and normal blood counts (one had 8 per cent eosinophils) and their serums did not show precipitins.

One of the most interesting aspects of the epidemic was the suggestion as to where the fungus could have been inhaled. The nature of the epidemic suggested that the infected students must have had a common massive exposure which the 4 negative to coccidioidin did not experience. The 3 coccidioidin positive faculty members might or might not have been exposed, previous studies having indicated that one infection apparently results in an immunity.

Not only is the mechanism of coccidioidal infection verified but also is resistance to exogenous reinfection substantiated. The incubation periods, nine to fourteen days, are likewise in accordance with the range previously established. Among the cardinal symptoms and signs summarized in the accompanying table, the pain or "tightness" in the chest is the most useful in a differential diagnosis. It is to be noted that none of the patients had erythema nodosum or multiforme.

Seven out of 14 university students and faculty members were infected with *Coccidioides immitis* on a field trip to a region adjacent to the San Joaquin Valley. All 7 and 2 of the others who apparently had been immunized by previous infection were subjected to a heavy dust exposure. The fungus was recovered from the soil which had been the source of the dust.

The infections of 6 of the 7 victims were accompanied by moderately severe symptoms which lasted from three to six weeks, but all patients recovered with out the development of coccidioidal granuloma.

EMMONS (1942) isolated *Coccidioides* from *soil* and *rodents.*

During the summer of 1941 coccidioides was isolated from 5 of 150 soil samples collected in the desert near the village of San Carlos, Arizona.

From information obtained during the attempts to isolate coccidioides from San Carlos soil it became increasingly apparent that the conception of coccidioides as a soil-inhabiting fungus must be revised. Coccidioides is a rapidly growing fungus and should be relatively easy to isolate from soil if it were growing freely as a saprophyte in this substratum.

A new approach was therefore made and when studies were resumed at San Carlos 105 wild rodents were trapped in a preliminary search for infected animals. Deer mice (Peromyscus), pocket mice (Perognathus), grasshopper mice (Onychomys), kangaroo rats (Dipodomys), pack rats (Neotoma), and a

rectis vel arcuatis, flexuosis, hyalinis, guttulatis, 2—5 septatis, non constrictis, raro in septis secendentibus, µ 22—32 ⩔ 2,5—3,5.

Hab. in foliis vivis Loranthi sp. (Loranthaceae), Oletta (Scioa), ca. 2400 m.s.m., 9/11/1938.

Il fungillo, assai comune in tutto l'ovest etiopico, produce macchie giallo-grigiastre, talora rossastre, sollevate sul lembo fogliare, che può esserne in gran parte coperto. Al centro di dette macchie, il parassita forma fruttificazioni sub-epidermiche, lenticolari, poi largamente erompenti. Le fruttificazioni mature sono talora più immerse, infundiboliformi o a coppa, talora invece del tutto appianate, con sporule disposte quasi verticalmente e parallele tra loro su uno strato proligero uniforme. Dette sporule, a profilo irregolare, cilindracee o più spesso fusoidee, talvolta si spezzano in due-tre parti in corrispondenza dei setti.

Il micete, ravvicinabile qualche volta a *Septoria* Fr., è forse più spesso a maturità vicino a *Septogloeum* Sacc. Esso è stato però riferito a *Phleospora* Wallr. per il diverso modo di presentarsi di fruttificazioni meno patenti e piuttosto immerse nei tessuti matricali.

In Argentina è stata segnalata da Spegazzini [in Anal. Mus. Nacion. Buenos Aires, vol. XX, pag. 384 (1919)] su *Loranthus ligustrinus* Wall. una *Septoria loranthicola,* riportata nella Sylloge (vol XXII, pag. 1112) con un punto d'interrogazione (*Septoria? loranthicola* Speg.). Benchè non sia stato possibile esaminare ,,exsiccata'' di questa *Septoria,* sembra tuttavia di dover notare che per comportamento, caratteri morfologici, dimensioni, la *Septoria loranthicola* e la presente *Phleospora* sembrano coincidere assai bene, per quanto può desumersi dalla diagnosi della prima.

Colletotrichum gloeosporioides Penz. in F. agrum., II, pag. 6 (1882); Sacc., Syll. fungor., vol. III, pag. 735. Su foglie di Citrus bigaradia Duh., Gumaro presso Gore, ca. 1800 m.s.m., 16/12/1938.

Scolecosporium syzigii n.sp.

Acervulis amphigenis, plerumque hypophyllis, subcutaneo-erumpentibus, epidermide fissa cinctis, pulvinatis, applanatis, atris, subrotundatis vel polygoniis, µ 300—600 diam., saepe late confluentibus; conidiis 2—6 locularibus, castaneo-fuscis, obtuse verruculosis, infra rotundatis, sursum longe attenuatis vel prorsus rostratis, ibique sub-hyalinis, µ 40—60 ⩔ 6—8; conidiophoris dense stipatis, cylindraceis, parum septatis, concoloribus, µ 20—30 ⩔ 6—8.

Hab. in foliis Syzigii guineensis DC. (Myrtaceae), prope fluminem Omo-Bottego, ca. 1700 m.s.m., 25/1/1939; socia Micronectriae syzigii.

La presente forma, diversa per caratteri morfologici dalle altre specie di questo genere, si riscontra anfigena, ma sopratutto sulla pagina inferiore fogliare, di *Syzigium guineense,* e in particolare sui margini delle macchie di secco nelle quali si riscontra la *Micronectria* precedentemente descritta; macchie che lo *Scolecosporium* sembra estendere. Gli acervuli spesso confluenti del micete attirano l'attenzione dell' osservatore per l'intensa tonalità scura e le dimensioni notevoli (anche di varii millimetri, se gli acervuli confluiscono). Lo stroma, di colorazione sfumante dal testaceo alla terra d'ombra, erompe largamente a maturità e si solleva sull' epidermide, i cui lembi lacerati lo cingono. Gli acervuli maturi sono pulvinati e mostrano superficie piana, vellutata. I conidiofori, che si sollevano dallo strato proligero sono strettamente raddicinati, diritti, cilindrici, unisettati alla base, talora due-tresettati, arrotondati all' apice. I conidii, 2—6 loculari, di solito bisettati, intensamente castanei, a base arrotondata, si sollevano verticalmente dai conidiofori sui quali si inseriscono per un ilo ben evidente. Essi hanno parete ornata di verrucule ottuse, sparse, particolarmente frequenti nella porzione mediano-basale, e, cilindracei per la metà o i due terzi inferiori, si attenuano gradualmente e lungamente all' apice, arcuandosi spesso in maniera evidente in questa porzione superiore. Questo *Scolecosporium* è in genere socio di *Micronectria syzigii* (vedi ivi).

Pestalozzia caudata Sydow in Bull. l'Herb. Boiss., II ser., vol. I, pag. 84 (1901); Sacc., Syll. fungor., vol. XVI, pag. 1017. Su brattee fiorali di Cyperus papirus L., sponde del Nilo Azzurro nei pressi del lago Tana, ca. 1850 m.s.m., 25/2/1939.

Secondo la descrizione di Sydow, la lunghezza delle spore raggiunge μ 36—45. Le misure date da Guba [Mycol., vol. XXIV, pag. 363 (1932)] invece sono inferiori (μ 28—36); e quelle date da Grove [Brit. stem and Leaf-fungi, vol. II, pag. 349 (1937)] lo sono ancora di più (μ 28). Nell' esemplare esaminato dunque le spore sarebbero eccezionalmente sviluppate, oscillando in dimensioni da μ 37 a 55 (moda su 100 misurazioni = μ 45). La perfetta corrispondenza dei diversi caratteri morfologici dell' esemplare esaminato con quelli assai singolari del tipo sembra però non lasciar dubbi sul riferimento specifico.

Pestalozzia theae K. Sawada in Mycol., vol. IX, pag. 171 (1917); Sacc., Syll. fungor., vol. XXV, pag. 607. Su foglie di Thea sinensis L., Bonga (Galla e Sidama), ca. 1750 m.s.m., 22/11/1938.

Pestalozzia versicolor Speg. in Michelia, vol. I, pag. 479 (1879); Sacc., Syll., fungor., vol. III, pag. 790. Su foglie di Protea abyssinica W., valle del fiume Diddessa (Galla e Sidama), ca. 1600 m.s.m., 3/12/1938.

Questa *Pestalozzia* si è formata su vecchie foglie, morte e scolorate di *Protea abyssinica*, parassitizzate da *Septoria proteae* n.sp. (vedi ivi). Le fruttificazioni pseudo-picnidiche, anfigene, subepidermico-erompenti, di μ 120—140, sono limitate da una parete stromatica, che le ricopre in gran parte. A maturità l'epidermide lacerata si solleva a tronco di cono; e le spore sono emesse in globulo atro, convesso, quasi emisferico. Queste (μ 20—28 ⩔ 6—7) sono quinqueloculari ed hanno cellula inferiore (μ 4) regolarmente conoidea e cellula superiore obconica o più raramente tronco-conica, coronata di solito da tre ciglia filiformi, non rigonfiate all' apice e divergenti (circa μ 18—22). I loculi mediani, misuranti μ 15—18, sono di diversa tonalità: l'inferiore di tenue colorazione fumosa, i due superiori olivacei, il mediano assai intensamente colorato. Nonostante non si abbiano senalazioni di questa *Pestalozzia* su *Protea* o su generi vicini, si è creduto di poter riferire ad essa in base a questi caratteri la forma osservata. *P. versicolor* Speg., riscontrata nel tipo su foglie di *Nerium Oleander* L. cadute e in decomposizione, è stata ritrovata poi dallo stesso [f. *vagans* Speg., in Rev. Mus. de La Plata, vol. XIV, pag. 42 (1908)] su foglie in decadimento di molti alberi e arbusti ed è stata inoculata da Klebahn [Myc. Cblatt., vol. III, pagg. 109—115 (1913); id., vol. IV, pagg. 109—115 (1914)] con successo su diverse matrici, ma solo sotto campana e non in serre aperte: per cui anche questo autore la ritiene saprofita polifago 1).

Ovulariopsis erysiphoides Pat. e Hariot in Journ. de Bot., vol. XIV, pag. 245 (1900); Sacc., Syll. fungor., vol. XVI, pag. 1936. Su foglie di Erythrina sp., Soddu (Galla e Sidama), ca. 2000 m.s.m., 27/1/1939; e su foglie di

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1) Guba però [Phytopath., vol. XIX, pagg. 22 (1929)] non ritiene esatto il riferimento specifico del materiale usato da Klebahn.

an illness characterized by malaise, cough, fever, rash, joint pains and nodules over the extremities?

The 17 cases who reacted to coccidioidin, 9 had lived in or visited one or more of the southwestern states. Only 8, or 1.2 per cent, of the remaining 659 boys who gave no history of residence in the southwestern states reacted to coccidioidin.

Of the 17 boys who reacted to coccidioidin, 12 failed to react to the intracutaneous injection of 0.01 and of 1.0 mg. of old tuberculin. Of this latter group 5 gave no definite medical history suggestive of coccidioidomycosis, although they showed definite calcified pulmonary nodules ranging in size from 2 to 8 mm. in diameter, located well out in the lung fields and in the hilar area; 4 of this group had at no time visited or lived in the Southwest. Of the 17 boys who reacted to coccidioidin, 2 gave a history of an illness suggestive of coccidioidomycosis and 8 had at no time been west of the Mississippi River.

Ashburn and Emmons (1942) reported on *spontaneous Coccidioidal granuloma in the lungs of wild rodents.*

Emmons recently reported the finding of fungi in 25 of 105 animals trapped in the desert around San Carlos, Arizona. His investigation was undertaken because of the demonstration by Aronson, Saylor and Parr that a high percentage of Indian school children on the San Carlos Indian Reservation were sensitized to coccidioidin and because the concept that *Coccidioides* is a soil-inhabiting fungus seemed inconsistent with difficulties in isolating it from soil. Of the 25 fungus-infected animals, 7 of the pocket mice (Perognathus), 1 kangaroo rat (Dipodomys) and 1 ground squirrel (Citellus) had gross lesions in their lungs at autopsy.

Of 105 rodents trapped in the desert around San Carlos, Arizona, 9 showed gross pulmonary lesions — 7 pocket mice (Perognathus), 1 kangaroo rat (Dipodomys) and 1 ground squirrel (Citellus). Microscopically, a total of twenty nodular lesions were found in the 1 mice and 1 kangaroo rat. The lesions occurred most often in the lower lobes of the lungs and particularly along the anterior border and in that portion of the lung occupying the costophrenic angle. Most of them were superficially located, and many caused slight elevation of the overlying pleura. Except for those in 1 animal (discussed in the text), most of the nodules measured 1 mm. in diameter. Basically each was formed of fusiform epithelioid cells, diffusely and irregularly disposed in the center but often showing concentric arrangement peripherally. A few lesions were formed centrally of adherent large mononuclear cells. Six nodules had large central zones of caseous necrosis, varying from 300 to 800 microns in diameter; one of these was partially calcified. Fibrosis was present in only two lesions, being of moderate degree in one and slight in the other. A few lymphocytes were present in the outer wall of most nodules, and three had sharply limited, densely cellular lymphocyte mantles, varying from 25 to 200 microns in thickness. The multiple small pulmonary lesions present in the ground squirrel were formed of grouped alveoli filled with sheets of large mononuclear cells. In all of the nodules there were present few to many fungus cells of varying size. In two nodules (2 mice) six fungi showed cytoplasmic cleavage or mature endospores typical of *Coccidioides.*

Stiles and Davis (1942) investigated the incidence of *Coccidioidal granuloma* (*Coccidioidomycosis*) in man and animals and its diagnosis in animals.

In 1931 Beck reported 286 human cases of chronic coccidioidal granuloma. Of these, 254 occurred in California and 16 in other states — Arizona, Colorado, Illinois, Kansas, Missouri, Nebraska, New Mexico, Pennsylvania, South Carolina, Texas and Washington; 14 cases occurred in South America and 2 in Italy.

Other localities reported by MILLER in 1936 are Alaska, Tennessee, Louisiana and Hawaii. EVANS and BALL report 1 human case in Old Mexico.

A new area of primary coccidioidal infection in the vicinity of Camp Roberts, California, is reported by SHELTON, who found 14 infected soldiers from among 736 recruits recently stationed at that camp. These infected men gave a negative reaction to the coccidioidin skin test on arrival. However, after three months' stay, their reaction to the test became positive.

In Texas, SMITH reports a case of coccidioidal infection occurring in a man living in the vicinity of El Paso who had never been in California. From Texas, also, CALDWELL reports 3 proved cases from the Dallas area and 3 more human infections since the publication of his article. At San Angelo, Texas, SCHULZE reports that 18 per cent of 100 private patients reacted to the coccidioidin skin test. All these reactors were ranchmen none of whom had ever visited the San Joaquin Valley. Three cases of the acute "Valley fever" type infection and 9 cases of the granulomatous disease arising in western Texas are mentioned by Schulze as having been reported by other observers.

In the vicinity of Phoenix, Arizona, PHILLIPS states that he found 10 per cent of the older residents of the Salt River Valley gave positive coccidioidin reactions. Many of these persons were from rural areas in contact with livestock, but, curiously enough, the largest incidence of positive reactors were found not among cattlemen but among men who handled sheep.

MILLS and FARNESS cite the case of a 15 year old boy attending a private school near Tucson, Arizona, who showed cavitation of the lungs on roentgen examination. The continued absence of the acid-fast organisms, high eosinophilia and general absence of fever prompted the more thorough examination of the sputum, which resulted in recovery of a fungus recognized as *Coccidioides immitis*. The finding of this positive case stimulated these physicians and a dozen or more clinical diagnoses came to their attention. Intradermic coccidioidin tests on 60 Arizona patients in the Pima County Hospital showed that 20 per cent gave a positive reaction. Similar results were obtained in other groups of patients. FARNESS cites 5 human cases apparently arising in Arizona.

The latest published articles and personal communications (1942) indicate that at least 790 cases of chronic coccidioidal granuloma have been recognized in human beings. Of this number, WYNNS, who is epidemiologist of the California State Board of Health, reports a mortality of nearly 40 per cent in 744 California cases of this highly fatal chronic form of the disease. His report includes BECK'S 254 cases.

The senior author (G. W. S.) has observed but 1 human case of coccidioidomycosis at the Colorado General Hospital, reported by O'TOOLE and originating from California.

While the greatest interest in occidioidal infections has centered in human medicine, the veterinary phase of the problem has its place in relation to public health and animal welfare.

A variety of animals may be sources of coccidioides infection. EMMONS of the U. S. Public Health Service recovered the fungus in 25 of 105 trapped rodents representing six species in the vicinity of San Carlos, Arizona. Spores of the fungus were also found in the soil of this locality, possibly arising from animal contamination.

From Tucson, Arizona, FARNESS reports a case of coccidioidal infection occurring in a great Dane dog. Later in a personal communication he cites 5 additional canine cases diagnosed either by cultural methods or by human coccidioidin applied as a cutaneous test. FARNESS also has 2 cats under observation in which he suspects coccidioidomycosis.

Another canine case occurring in a 2 year old male English setter is reported in Canada by PLUMMER and RADMORE. The dog in question was raised in Canada and was mated with a female setter from California. In sections coccidioidal spherules were demonstrated in large numbers in the lung and brain tissues. Although the lungs grossly resembled tuberculosis, evidence of acid-fast organisms was not demonstrated.

While engaged in the diagnosis and treatment of human coccidioidal infections in Arizona, PHILLIPS called attention to a condition in sheep resembling coccidioidal granuloma.

In a band of 56 sheep kept for three years near Phoenix, 30 head gave positive reactions with 0.1 cc. of human coccidioidin administered under aseptic conditions. After this several sheep became ill with temperatures of 105 F. or higher, and in 2 abscesses developed in the axillary lymph nodes. Aspirated pus from the softened lymph nodes was cultured, resulting in the recovery of a fungus-like organism which on inoculation killed male guinea pigs with septicemia. The tentative conclusion was that a variant of *Coccidioides immitis* was present in the Phoenix district, that it affects sheep in particular and that it produces a nonfatal, usually undiagnosed illness in human subjects. He further states that open ulcers persisted for one year on three of the sheep mentioned. These sheep were destroyed without further laboratory examination.

BECK in 1929 reported a single positive case of coccidioidal granuloma in sheep.

During the past ten years 78 positive cases of coccidioidal infection in bovines were diagnosed in the laboratory of the Denver Bureau of Animal Industry which originated from localities as follows: California 62, Arizona and Texas 5 each, Old Mexico 4, Colorado and New Mexico 1 case each. These definitely diagnosed cases represent many additional cases in lots sent for slaughter when only representative samples from a lot were sent to the laboratory for examination. For example, in one lot 6 specimens were submitted with the statement that 45 steers were similarly affected. In another instance a single specimen was submitted with the notation that 36 steers from the San Joaquin Valley showed similar lesions. With few exceptions these tissues were from cattle slaughtered at federally inspected abattoirs. Their origin and probable source of infection were traced through shipping records in practically all instances. This group consisted of 32 male animals, 33 females and 13 in which the sex was not given.

Two 8 to 10 month old calves were the youngest animals noted that showed coccidioidal infection. Thirty-nine cattle were from 10 months to 3 years old, 17 were past 4 years and the ages of the remaining 22 were unknown. The lesions were distributed as follows: bronchial lymph node only in 21 animals; mediastinal lymph node only in 21 animals; bronchial and mediastinal lymph nodes in 13 animals; pulmonary lesions with lymph node involvement in 8 animals and in 15 animals the location of the lesions was unidentified.

In 1918 GILTNER reported the first cases of coccidioidal granuloma in bronchial and mediastinal lymph nodes from cattle slaughtered at San Diego, California. From these tissues *Coccidioides immitis* was recovered on culture, and pathogenicity was detemined by successful experimental injections into guinea pigs, rabbits, dogs, cattle, sheep and swine.

During 1931 BECK reported 10 cases of coccidioides infection occurring in cattle in addition to 9 other bovine cases previously reported. During the period from Dec. 17, 1938 to Feb. 6, 1939, BENGSTON, in charge of the bureau's pathologic laboratory at Chicago, diagnosed 20 cases of coccidioidal granuloma from California and Texas cattle aged from 1 year upward. Steers, cows and bulls were affected. In 16 cases the mediastinal lymph nodes only were involved; in 3 cases the bronchial lymph nodes and 1 case the bronchial lymph nodes and lungs were infected.

From the bureau's laboratory in Washington, D. C., SCHOENING reports several positive cases of coccidioidal granuloma during recent years. A summary of the data now at hand shows a total of 116 definitely diagnosed cases of bovine coccidioidal granuloma in the United States. However, it is probable that many more cases in cattle have escaped identification or were not submitted for laboratory confirmation.

Microscopic study of sections of the lesions stained with hematoxylin-eosin show a granulomatous process consisting of connective tissue, blood vessels, lymphocytes, plasma cells, mononuclear and polymorphonuclear leukocytes, a few eosinophils and giant cells. Scattered throughout the tissue are seen variable numbers of double contoured spherules the majority of which have been taken up by giant cells. Usually a single parasite is seen within a giant cell, but as many as six spherules have been noted within a single giant cell in some sections. Some of the parasites, which are presumably the more recent sporulating forms, often show a strong affinity for eosin. Occasional spherules show ruptured walls, and in some sections one or more parasites may be surrounded by a group of eosin-staining radiating clubs forming a well defined rosette.

SHELTON (1942) made a survey of *Coccidioidomycosis* at Camp Roberts, California.

In the early months of the development of Camp Roberts, among the many cases of acute respiratory illness receiving treatment at the Station Hospital, 2 cases of primary coccidioidomycosis were discovered. Camp Roberts lies on the western slope of the Coast Range Mountains of California, separated from the San Joaquin Valley by that range, and since this area had not previously been known to harbor the disease as an endemic focus a survey was undertaken to determine the extent of the disease locally, the results of which survey constitute the body of this report.

This report is based chiefly on the results of two skin test surveys made three months apart, the two surveys being made on the same group of persons, with a view to finding in how many instances the test became positive during the three month interval. In addition, there are reports of 3 cases discovered in the hospital — not a part of the survey.

The two series of tests were made on a battalion of men plus a few men from three other battalions newly arrived at Camp Roberts. The first test series was done June 21 on a battalion of troops that had arrived between June 12 and June 15. The troops were gathered from the Middle West — Illinois, Missouri, Nebraska, Arkansas and Texas — and no attempt was made to list them by residence except for those who reacted positively to the first test.

The second series of tests was made on the same men on September 13 after three months of training in and about Camp Roberts and just before their transfer to other camps. All reactions were read at from forty-four to forty-eight hours and were recorded thus:

definite induration and erythema but less than 1 cm. in diameter.
induration of 1 cm. in diameter.
induration of 1 cm. in diameter plus flare of erythema of 1 cm. or more.
induration of 2 cm. or more.
vesiculation.

Those reacting positively to the second test who were previously demonstrated to be nonreactors were questioned regarding symptoms during the three months interval, and roentgenograms of the chest were made.

TABLE I. *Results of Coccidioidin Skin Tests*

	Number
First test, June 21, 1941	
Men tested	888
Positive	11
Second test, Sept. 13, 1941	
Men tested (less positives from first test)	736
New positives	14

TABLE II. *Previous Residence of Men Found Positive to Coccidioidin on Arrival at Camp Roberts*

Case	Reaction	Past Residence in Known Endemic Areas
C. M.	+	Bakersfield (San Joaquin Valley).
S. D.	++	Fresno (San Joaquin Valley)
R. S.	++++	Exeter (San Joaquin Valley)
I. C.	++	Texas
J. N.	+++	Texas
L. G.	±	Texas
R. R.	+++	Texas
R. M.	+	Texas
K. C.	±	Texas
J. R.	++	Arkansas only
C. B.	++	Idaho and Montana only

All were carefully questioned regarding visits to other localities during this time.

The first series of tests was accomplished on 888 men. Eleven were found to give a positive reaction, though they had been at Camp Roberts too short a time to have picked up the disease here. Of these positive reactors, 1 had lived only in Montana and Idaho, 1 had lived only in Arkansas not far from Texas.

TABLE III. *Trips to Other localities Made by Men Developing Positive Reaction While at Camp Roberts*

	No. of Cases
Visit to San Joaquin Valley	3
Visit to Los Angeles	4
Visit to San Francisco	3
No farther than Salinas (78 miles north)	1
No farther than Pismo Beach (50 miles south)	1
No farther than Paso Robles (15 miles south)	1
No farther than San Miguel (3 miles south)	1

Six had lived in Texas and 3 had lived in the San Joaquin Valley of California. Texas and California are already well recognized endemic foci of the disease, and the reaction in the first case suggests the possibility that Montana or Idaho may harbor the disease.

The second series included only 736 of the 877 who gave a negative reaction to the first test, because many of the men were not available, being on leave or having been transferred. Actually, of the 11 found positive in the first test, 8 were retested and all reacted positively the second time. Of the 736 men in the second series, all negative to the first test, 14 gave positive reactions. In other words, 14 men proved to be nonreactors in June reacted positively in September. Three of these had visited in the San Joaquin Valley during their stay at Camp Roberts, 7 had been to Los Angeles or San Francisco and 4 had not left the vicinity of Camp Roberts more than a few miles, having gone to Salinas (1 case), Pismo Beach (1 case) and Paso Robles (2 cases).

Of the 14 positive reactors, only 1, according to a check in the hospital records and answers to a questionnaire, had entered the hospital for any febrile or respiratory illness, though hospitalization is the routine procedure for any illness severe enough to prevent exercise of full duty. It is obvious that the onset of the infection was not attended by the development of distressing symptoms in most of the cases.

The chief significance of the investigation here reported is the demonstration of the fact that coccidioidomycosis can be acquired in the environs of Camp Roberts, California, a hitherto unknown endemic focus. This fact has been proved by the finding of 14 persons who were known to have given negativ reactions to the coccidioidin skin test on arrival here and positive reactions three months later.

By extrapolation, if it is assumed that the percentage of men found infected is uniform throughout the camp, it could be estimated that, of the 20,000 to 25,000 men here, from 400 to 500 acquired primary coccidioidomycosis between June and September of this year. Observations elsewhere indicate that the incidence of new cases is highest in the dry months, falling to almost nothing in the wet winter months, so that the number of cases developing in three months of the summer represents nearly half of the annual incidence.

BAKER and SMITH (1942) investigated the *utilization* of *carbon* and *nitrogen compounds by Coccidioides immitis* (Rixford and Gilchrist, 1896).

Very little information is available concerning the assimilation of carbon and nitrogen compounds by *Coccidioides immitis*. All investigators have agreed that carbohydrates are not fermented with the production of acid or gas. STEWART and MEYER demonstrated that glucose is utilized by the fungus but could not detect the presence of incompletely oxidized end products.

HIRSCH and BENSON demonstrated that ammonium lactate can serve as a source of carbon. STEWART and MEYER found that acetic acid, but not formic acid, can serve as a carbon source and that amino acids, but not urea, can serve as both carbon and nitrogen sources.

Many investigators have demonstrated the liquefaction of gelatin by *C. immitis*. According to MOORE and HIRSCH and BENSON, milk is coagulated and peptonized. CASTELLANI and JACONO and CIFERRI and REDAELLI, on the other hand, found that milk is neither coagulated nor digested. According to CIFERRI and REDAELLI, keratin (hair and feathers) is digested.

CIFERRI and REDAELLI stated that *C. Immitis* will not grow well in the absence of complex organic nitrogen compounds. They obtained good growth on peptone, Liebig's meat extract and powdered blood mediums but poor growth on synthetic mediums containing ammonium sulfate, potassium nitrate or nitrite, asparagine and glycine as nitrogen sources. However, Hirsch and Benson found that the fungus grows well in a medium containing ammonium lactate as a source of both carbon and nitrogen. Moore obtained good growth of the fungus on Czapek's agar with nitrate as a source of nitrogen. Stewart and Meyer obtained good growth with ammonium salts, acetamide and amino acids, fair growth with urea and poor growth with nitrates as sources of nitrogen. A basal medium having the following composition was prepared:

Potassium phosphate (primary) . . .	2.0 gm.
Potassium phosphate (secondary) . .	2.0 gm.
Magnesium sulfate	0.5 gm.
Ferric chloride	0.1 gm.
Distilled water.	1000 cc.

A wide variety of relatively simple carbon and nitrogen compounds can serve as sources of carbon and nitrogen for *Coccidioides immitis*. The carbon sources include: many sugars, alcohols, organic acids, amino acids and amides. The nitrogen sources include: peptone, amino acids, amides, ammonium ion, and nitrate ion.

Emmons and Ashburn (1942) isolated *Haplosporangium parvum* n.sp. and *Coccidioides immitis* from *wild rodents* and investigated their relationship to coccidioidomycosis.

The study was made on the San Carlos Indian Reservation, San Carlos, Ariz., which lies outside the previously recognized endemic areas of the disease. This area was chosen because the studies of Aronson et al. had furnished evidence that coccidioidomycosis is prevalent there, although not recognized clinically. This evidence was based on the demonstration that 92 percent of Indian school children tested reacted to the intradermal injection of coccidioidin. The small rodents were first investigated because they are very numerous on the desert, and some species were known to be susceptible to experimental infection. During December 1941, and March, April, and July, 1942, 303 wild rodents were trapped and examined (table 1). They were obtained from five sites in an area surrounding San Carlos Hospital, within a maximum radius of about 7 miles.

Table I. *Animals with fungus infections as determined by observation of lesions or isolation of cultures, tabulated according to species and time of collection.*

	December Series		March Series		July Series		Total		
	Trapped	Infected	Trapped	Infected	Trapped	Infected	Trapped	Infected	Percent Infected
Perognathus bailevi (pocket mouse)	12	9	10	10	6	6	28	25	89
Perognathus penicillatus, P. intermedius (pocket mice)	12	11	73	57	11	10	96	78	81
Dipodomys merriami (kangaroo rat)	7	3	19	9	3	0	29	12	41
Citellus harrisi (squirrel) . .	1	1	8	7	1	0	10	8	80
Onychomys torridus (grasshopper mouse)	10	0	10	2	7	1	27	3	11
Peromyscus eremicus (deer mouse)	59	2	48	0	6	0	113	2	1.7
All species	101	26	168	85	34	17	303	128	42

TABLE II. *Species of fungus isolated from rodents with and without observed lesison*

	Total Trapped	Lesions	Only *C. immitis* isolated	Only *H. parvum* isolated	*C. immitis* and *H. parvum* isolated	Total animals
Perognathus	124	Seen	11	18	5	34
		Not Seen	1	60	2	63
Dipodomys	29	Seen	3	1	2	6
		Not Seen	—	5	—	5
Citellus	10	Seen	—	5	—	5
		Not Seen	—	—	—	—
Onychomys	27	Seen	1	—	—	1
		Not Seen	—	1	—	1
Peromyscus.	113	Seen	—	—	—	—
		Not Seen	—	2	—	2
Total	303		16	92	9	117 *

* Cultures from 11 additional animals were contaminated and the pathogens, if present, were lost.

Of the 303 wild rodents trapped, 128, or 42 percent, had fungus infections (table 1). This number represents a selection. It was found early in the study that a mycosis was frequently present in certain species of rodents, and in subsequent studies attempts were made to trap as many as possible of these species. Lesions were observed in 55 animals and fungi were isolated from 46 of these. Fungi were also isolated from 71 in which no lesions were found (table 1 and 2). The fungus isolated was *C. immitis* in 25 cases and an apparently related new fungus described below in 101 cases (table 2). These numbers include 9 cases in which both fungi were isolated from the same animal. The strains of *C. immitis* isolated were typical of the species in morphology and virulence for laboratory animals and need not be considered further at this time. The second fungus requires a description, a recital of the circumstances surrounding its isolation, and a discussion of its probable relationship to the disease in rodents and to *C. immitis*. This fungus has the generic characters of *Haplosporangium Thaxter*, and the name *Haplosporangium parvum* is proposed, the specific name being descriptive of the small size of the fungus in comparison with other species of the genus. *Haplosporangium parvum sp. nov.: Mycelio tenue, albido, demum fulvello; conidiophoris gracilibus, indivisis vel ramosis,* 0.5 × 1—10μ; *conidiis globosis, rugosis vel levibus,* 3—3.5μ diam. *Mycelium delicate,* matted, white, becoming slightly brownish with age, aerial hyphae tufted in some strains which produce unusually large numbers of spores, sparse in other strains; hyphae averaging about 1μ, but rarely reaching 4μ in diameter, with few septa and these not necessarily related spatially to branches; conidiophores slender, simple and only 0.5–4μ long, or branched and 0.5 × 1–10μ, in most cases not tapering toward the apex; conidia spherical or subspherical, minutely spiny, becoming smooth, 3—3.5μ in diameter, containing one or more hyaline bodies. Present as spherical nonbudding cells reaching a diameter of 14μ in lung tissue of *Perognathus, Dipodomys, Citellus,* and other rodents trapped in the vicinity of San Carlos, Ariz. *Haplosporangium parvum* grows more slowly than *C. immitis*. On acid dextrose agar the colony appears first as a glabrous colorless disc, the hyphae forming a rather firm surface mat with an even border. As growth proceeds, a small tuft of white aerial hyphae appears at the center of the colony, and delicate aerial hyphae gradually appear over the rest of the colony. The strains isolated vary considerably in colony characteristics. Some always retain glabrous zones. Most become entirely covered with densely matted but delicate white aerial hyphae, becoming brownish with age, which may form a cottony layer as much as 6 or 8 mm. deep. On agar slants this extends up onto the sides of the culture tube. In some strains the colony surface becomes tufted with a suggestion of chalkiness and is cream colored even while still young, a condition usually associated with increased spore production. Such strains, approach *C. immitis* in appearance, but the hyphae are always more delicate and the microscopic morphology is different.

Microscopic. — The hyphae are delicate, most falling within the limits of 0.5—2μ and averaging about 1μ in diameter. Occasional hyphae reach a diameter of 4μ. Branching is frequent and the location seems to be independent of the location of cross walls. Septa are infrequent or difficult to demonstrate except in old hyphae, where the protoplasm is sometimes divided by pseudosepta and vacuoles in a manner suggesting the *Phycomycetes*. The spores are borne singly, or rarely in chains of two at the ends of hyphae or at the tips of short lateral conidiophores. *Conidiophores* are simple (0.5 × 1—10μ) or complex The latter vary from short branches bearing two or more spores to longer branches bearing many lateral spores. A swollen hyphal tip which would appear to have the potentialities of a spore proliferates by the formation of two or more branches which are terminated by conidia. *Conidia* are almost spherical except for a short tubular projection or stalk at the base. Many young spores are slightly flattened, the point of attachment to the conidiophore being on one flattened side. Spores average 3—3.5μ in diameter. Exceptional spores are clavate and much larger. Spore formation is initiated by the development of a smooth spherical swelling at the tip of the conidiophore. As this swollen structure increases in size, rough markings, which under high magnification resemble spines, develop on the outer surface. In mature spores these markings are less conspicuous, and many old spores are quite smooth. Formation of the spore is completed by the development of a wall across the conidiophore 0.25—0.5μ below the tip of the latter. The location of this wall well below the spherical portion of the spore produces the stipitate character of the spore already mentioned. The manner of spore development in culture may be interpreted as an endogenous process within a sporangium. Shrinkage of the immature sporangium when placed in mounting fluid for examination probably emphasizes the external markings. As the spore matures it increases in size, completely filling the space, and the sporangial wall disappears, or more probably, adheres closely to the spore wall, the external markings being lost or partially obliterated. The structure can be defined as a monosporial sporangium or sporangiole, or more properly, a conidium. If this interpretation is correct, the fungus may be placed beside the three species of *Haplosporangium Thaxter* already described. Its spores also resemble the "stylospores" of *Mortierella*, furnishing additional evidence for a relationship to the *Phycomycetes*. It differs from the other species of *Haplosporangium* in size and shape of the conidiophore. *H. bisporale Thaxter* and *H. decipiens Thaxter*, isolated from dung, and *H. lignicola* Martin, isolated from rotten wood, are much larger and have tapering conidiophores. Additional evidence indicating a probable relationship of *H. parvum* to coccidioidomycosis is furnished by the use of skin test. A skin testing antigen was prepared from *H. parvum* following the procedures used in making coccidioidin. This material was used in skin testing Indian school children at San Carlos and at Fort Apaches, an adjacent area in which only about 15 percent of the school children react to coccidioidin. The results of this testing are shown in table 3.

This probable antigenic relationship, the circumstances under which H. parvum was isolated from rodents, resemblances of the two fungi in lung

TABLE III. *Reactions of school children in two contrasting areas, injected intradermally on one arm with lot No. 9 of coccidioidin (Dr. C. E. Smith), and on the other arm with C. 2.*

	125 children tested at San Carlos		123 children tested at Fort Apache	
	Positive to coccidioidin 1 : 1000	Negative to coccidioidin 1 : 1000	Positive to coccidioidin 1 : 1000	Negative to coccidioidin 1 : 1000
Positive to C.2 1 : 100	27	2	2	2
Negative to C.2 1 : 100	65	31	5	114

tissue, and the results of laboratory investigations lead to the conclusion that *H. parvum* is genetically related to *C. immitis* and in some manner to coccidioidomycosis. If despite great morphological differences in culture, *H. parvum* is genetically related to *C. immitis,* it provides for the latter a hitherto unrecognized point of attachment to the *Phycomycetes.* It may be pointed out further that there is a remarkable resemblance in culture between *H. parvum* and two other pathogenic fungi, *Blastomyces dermatitidis* and *Histoplasma capsulatum.* The discovery of this fungus therefore supplies evidence which may indicate a closer relationship than has been previously recognized between the etiological agents of coccidioidomycosis, blastomycosis, and histoplasmosis, and between these *Fungi Imperfecti* and the *Phycomycetes.*

CARTER (1942) reported on *roentgen diagnosis* of *fungous infections* of the *lungs* with *special reference to Coccidioidomycosis.*

After the primary invasion, a number of patients, proportionally small but large in the aggregate, are destined to develop the serious secondary form of Coccidioidal Granuloma. The factors of this liability are not yet clear. Primary involvement predominates in the female, and appears not to be related to race, social status, or occupation. The secondary form shows a marked predilection for Mexicans, Negroes, Filipinos, and Chinese, of male sex, with occupations entailing special contact with soil or its products.

Coccidioidal granuloma may be either an acute progressive disease, fatal in a few weeks or months, or it may be chronic and protracted, with few or isolated foci of infection, ending in apparent recovery or disappearance of the patient from observation in relatively good condition. The chronic type may become acute, but many known fatal cases were acute from the beginning.

Extrapulmonary manifestations have been present in a large majority of the cases, and add so significantly to the likelihood of the disease that they should be mentioned before describing the pulmonary findings. These include enlargement of superficial lymph nodes, superficial or deep soft tissue abscesses, granulomatous nodules, ulcers or sinuses from adjacent infection in the skin, meningitis, and lesions of bones and joints. These tend to be multiple. Osseous and articular involvements are particularly suggestive.

Mediastinal adenopathy is the outstanding and most frequent single manifestation seen on the film.

Thus, the great majority of the fatal coccidioidal granulomas have been associated with pulmonary as well as lymph node involvement.

Miliary manifestations are evident in approximately a third of the cases.

An osseous lesion evident on the film of the chest remains a prominent manifestation, and occurs in about one-fourth of the cases. These are seen in ribs, scapulae, clavicles, and spine. Such lesions have been described elsewhere and will be discussed in another paper in this symposium. They add so significantly to the possibility of *Coccidioides* infection that, with any support otherwise, a provisional diagnosis seems justifiable.

Tuberculosis: Most of the roentgen appearances described above are compatible with a diagnosis of tuberculosis. The major predilections of the granuloma are minor ones for tuberculosis. Manifestations particularly suggestive of the mycosis are: the adenopathy, with or without the various parenchymal lesions; sparsity of cavities; associated osseous lesions.

Lymphomatoid Diseases: VIETA and CRAVER found nothing distinctive between the pulmonary manifestations in the lungs in HODGKIN'S desease, lymphosarcoma, and lymphatic leukemia. Mediastinal and hilar enlargements and infiltrative parenchymal lesions were frequent.

Pulmonary sarcoidosis may occasionally resemble coccidioidal granuloma. Both may have mediastinal enlargements, with or without miliary lesions, scattered nodular foci, or infiltrations radiating from the hila. Sarcoidosis appears to have a more symmetrical appearance in the two lungs than would be seen, except occasionally, in the mycosis. Sarcoidosis may have cutaneous lesions, from which the diagnosis may be made microscopically, and the clinical symptoms will usually be less severe. While both diseases may have cystic lesions in the bones of hands and feet, those of coccidioidosis will usually show frank evidence of infection, with sinuses, while those of sarcoidosis are indolent and may be clinically silent.

Bronchogenic carcinoma must occasionally be distinguished from coccidioidal granuloma. Either disease may show enlargement of the lymph nodes and non-characteristic parenchymal lesions, some of which may be miliary. The pneumonitis or neoplastic consolidations of the carcinoma may resemble the parenchymal lesions of the mycosis. Both may have adenopathy without an obvious parenchymal lesion. Cavities in the carcinoma are usually abscess-like, located in a tumor of the peripheral type, and may have a fluid level. Those of the mycosis are more tuberculosis-like.

Other fungous infections of the lungs, like coccidioidal granuloma, are relatively uncommon, and comparisons by large series, roentgenographically studied, are not usually available. Broad predilections only can be cited. Most mycoses are essentially granulomatous, having a varied tendency to persistent organized lesions: abscess, cavities, fibrosis, metastatic spread, invasiveness, and adenopathy. Some may be either chronic or acute, and some have a special tendency to protracted indolent involvement. As in coccidioidomycosis, one can readily list manifestations of the disease which have occurred. Close differentiation is not available.

Geographic considerations are important. *Coccidioidomycosis* is prevalent in California, centering about the San Joaquin Valley. Secondary centers occur in Texas, Arizona, and New Mexico. *Blastomycosis* is prevalent in the central and southern states. *Actinomycosis* is associated with rural conditions. Climate, and even its yearly variations, are a factor.

Blastomycosis is less frequently generalized than coccidioidal granuloma and less prone to profuse metastasis. When this happens, multiple osseous lesions may be found, which are indistinguishable from those due to *Coccidioides*. Pulmonary involvement includes miliary, nodular, and nodal lesions; confluent infiltrations and consolidations; cavities, air-containing or filled with necrotic material; and fibrosis, which may be diffuse or may occur in sheets, strands, or masses. Adenopathy and organized pleural lesions are common.

Blastomycosis appears to have a greater tendency to fibrosis than coccidioidomycosis, and less to adenopathy and miliary involvement. No absolute roentgenologic distinction appears to be available.

Actinomycosis: The primary infection may be pulmonary, cutaneous, oropharyngeal, or in the digestive tract. About 15 per cent of cases are found to involve the thorax. The disease is characterized by marked invasiveness, only moderate metastatic tendencies, fibrosis, and abscess formation. The involvement may be either primary or secondary.

Moniliasis: *Monilia* is so commonly a secondary or incidental invader in other diseases, and found so frequently with minor symptoms and radiographic findings, that the diagnosis of clinical moniliasis tends to be controversial. Rigid elimination of other infection, persistence of *Monilia* in the sputum, and careful prevention of contamination of examined specimens are required. The cases are usually mild, but may be severe and have been fatal.

Pulmonary torulosis is relatively infrequent. Pulmonary lesions of various kinds have occurred, including miliary foci, nodulations, consolidations, abscess, and thickening of peribronchial structures. A terminal meningitis is common.

Streptothricosis. Involvement in the thorax in streptothricosis is usually pulmonary in origin; and the disease may be acute or chronic.

Consolidations, abscess, bronchopneumonia, pleuritis, miliary and nodular lesions, have been found. The disease may resemble tuberculosis or other severe fungous diseases, particularly actinomycosis.

Miscellaneous bronchomycoses, usually chronic, include, among others, infections by *Aspergillus*, *Penicillium* and *Mucor*. Their predilection is to chronic involvement, with interstitial fibrosis, emphysema, bronchiectasis, and peripheral peribronchial abscesses. These have been described well by FAWCITT. He found a mixture of the three to be associated frequently with exposure to moldy hay; *Penicillium* especially with moldy grain; and *Mucor* with exposure to manure and soil.

J. Paracoccidioidal granuloma

DE ALMEIDA and DA SILVA LACAZ (1940) described a case of *Paracoccidioidal granuloma* in form of a severe stomatitis with enlargement of the cervical glands. Some sections of the oral mucosa showed a large number of giant cells and phagocytosed parasites.

DE ALMEIDA (1938) reported on the use of a *vaccine* against *Paracoccidioidal granuloma.* The vaccine was prepared from the contents of a swollen, softened lymph gland from which the *Paracoccidioides braziliensis* was shown in smears and in culture. This vaccine was employed in nine cases illustrated with case histories. The vaccine caused great improvement of the condition.

DE ALMEIDA, LACAZ, and FAVA NETO (1942) published statistical data on *Paracoccidioidal granuloma* in *Brazil.* This study is based on the material of the Archives of the Department of Microbiology of the School of Medicine of the University of Sao Paulo. They came to the conclusion that this mycosis is progressively spreading not only all over the State of Sao Paulo, but also all over Brazil and in South America as a whole. They found 570 cases of this mycosis in Brazil as of the 15th of April 1942. They revealed the following distribution:

Sex:	male 498, female 52, sex not given 20
Social status:	married 256, bachelor 157, widow 22, status not given 135

Age:	0 to 10 years	6
	10 to 20 ,,	77
	20 to 30 ,,	129
	30 to 40 ,,	100
	40 to 50 ,,	100
	50 to 60 ,,	66
	60 to 70 ,,	26
	70 to 80 ,,	4
	Without reference	62

Occupation: laborers 226, without reference 158, others 186

Origin: Sao Paulo 442, Rio de Janeiro 46, without reference 27, Minas Gerais 28, Baia 8, Pernambuco 7, Mato Grosso 6, Amazonas 1, Goias 1, Espirito Santo 1, Parana 3.

Nationality: Brasilian 289, Japanese 104, Spanish 68, without reference 25, Portuguese 30, Italian 28, Syrian 6, German 2, Hungarian 2, others 16, Tyrolese 1, Rumanian 4, French 1, English 1, Lithuanian 5, Russian 3, and Austrian 1.

De Almeida (1938) reported on observation of cultures of *Paracoccidioides cerebriformis*, Moore, 1935.

The mycologic studies made by Morris Moore in 1935 with the isolated fungus of Brazilian cases of blastomycosis, and kept as *Coccidiodes histoporocellularis* at the Mycothec of the Institute Oswaldo Cruz, led this mycologist to establish two facts: first, that these fungi belonged to the genus *Paracoccidioides* and not *Coccidioides* and, second, that a new species ought to be created. Thus appeared the species *Paracoccidioides cerebriformis* Moore, 1935. After a few transplantations, attention was called to the following curious fact: some of the cultures presented, in Sabouraud glycose agar, a red pigment that, in a few samples, invaded the whole colony and in others, only part of it.

After some time this red pigment disappeared and, when a new transfer was made 3 months later, the colony was completely white. Beside this different coloring, certain cultures showed macroscopically a different morphological aspect. One presented colonies with very pronounced furrows. The colony was easily detachable from the medium. The color of the colonies of this type is cream, sometimes tending to grey. The second type of colonies is white and they present larger circumvolutions. They develop a very light down. The colonies are more resistent, do not detach easily from the culture medium.

Connant and Howell (1941) investigated the etiological agents of *North* and *South American Blastomycosis.*

Although the stages in the life cycle of *Blastomyces dermatitidis* and *Paracoccidioides brasiliensis* have been studied by numerous investigators, and *Paracoccidioides* has been compared with *Coccidioides*, no detailed comparative study of the various forms of *B. dermatitidis* and *P. brasiliensis* has been made. In order to establish the identity of the South American fungus the present study was undertaken.

Seven strains of *Blastomyces*, isolated from lesions of North American blastomycosis, and 6 strains of *Paracoccidioides*, isolated from lesions of the South American disease, were compared as to their appearance in tissue, and their growth and microscopic appearance on various artificial culture media both at room and at incubator temperature. *Blastomyces dermatitidis* was seen in the tissues as singlebudding, thick-walled, yeast-like organisms. When diseased tissue or pus from blastomycotic lesions was placed on SABOURAUD'S glucose agar, after varying periods of time at room temperature, the fungus developed into a cottony-mold-like growth. Microscopically this growth was composed of a filamentous, branching, septate mycelium with numerous spores borne sessile or on short pedicles from the hyphae. In old cultures these spores developed into large, thickwalled, variously sculptured chlamydospores. When this cottony mycelium was transplanted to blood, glycerine, SABOURAUD'S dextrose, beef infusion or beef extract agar and incubated at 37° C. after varying periods of time the resulting growth was smooth and waxy or cerebriform and wrinkled, and lacked the aerial, mycelial growth typical of the cultures maintained at toom temperature. Microscopically this growth consisted of yeast-like, budding forms similar to those seen in diseased tissue or pus.

Paracoccidioides brasiliensis was seen in diseased tissues as multiple-budding, thick-walled, yeast-like cells. As in *Blastomyces,* when diseased tissue, obtained from experimental animals, containing this organism was placed on SABOURAUD'S dextrose agar, after varying periods of time at room temperature, the organism developed slowly, producing either cerebriform cultures similar to *Achorion Schoenleini* or cottony cultures with well-developed aerial hyphae. Microscopically these cultures were composed of a filamentous, branching, septate mycelium with intercalary, lateral and terminal chlamydospores. When this growth was transplanted to blood, SABOURAUD'S dextrose, beef infusion or beef extract agar and incubated at 37° C. after varying periods of time the fungus converted to a waxy and smooth or cerebriform growth. Microscopically this growth was composed of multiplebudding, yeast-like cells, identical with the forms seen in tissue.

Since these 2 fungi behave in a similar manner in tissues, in cultures at room temperature and in cultures at incubator temperature, it would seem that the differences noted above should be considered of specific rather than of generic importance. It is the writers' opinion, therefore, that only a single genus should represent the several isolations of fungi from North American blastomycosis and the several isolations of fungi from South American blastomycosis. For this reason the new combination, *Blastomyces brasiliensis,* is proposed for the fungi which produce South American blastomycosis. Although *Blastomyces* is not a suitable name for this genus because of the rules of priority in botanical nomenclature, it should be retained for these fungi until some generally accepted name is agreed upon. As only one species causes North American blastomycosis and the various species reported from South American blastomycosis differ only slightly in cultural aspects and morphology, only 2 species need to be considered, namely, *Blastomyces dermatitidis* Gilchrist and Stokes 1898 and *Blastomyces brasiliensis* (Splendore) Conant and Howell, comb. nov.

DE ALMEIDA and LACAZ (1942) discussed the value of the intradermal reactions in mycosis. They investigated the results of the skin reactions with trichophytin, levurine (Ravaut and Rabeau), actinomycetine (*Actinomyces bovis*) and paracoccidioidine, (*Paracoccidioides brasiliensis*). They came to the conclusion that all these reactions have no absolute value, because they may be also negative in presence of an existing, florid mycosis.

DE ALMEIDA and DA SILVA LACAZ (1940) described a case of *Paracoccidioidal granuloma* in form of a severe stomatitis with enlargement of the cervical glands. Some sections of the oral mucosa showed a large number of giant cells and phagocytosed parasites.

K. Histoplasmosis

De Monbreun (1939) reported of a case of *Histoplasmosis* in the dog as a natural host for *Histoplasma capsulatum.*

Histoplasma capsulatum has never been found free in nature and, up to the present time, man was its only known host. At the time Darling regarded the parasite as a protozoon he searched extensively for other natural hosts in the Canal Zone. In this study Darling examined the intestinal tracts of various insects, including those of larvae and adult mosquitoes. Also he examined the feces of calves, iguianas, raccoons, monkeys, snakes, marsupials, mice, and rats that were received in his laboratory. The only parasite obtained, which he regarded of significance, was a flagellated protozoon found in bloody mucus from the colon of a rat that was killed in his laboratory courtyard. Darling thought that this parasite might be a flagellated phase of *Histoplasma capsulatum.*

The case of natural histoplasmosis in a dog occurred in a three-year-old male, Boston Bull terrier. The dog, born and reared in Nashville, Tenn., appeared to be in good health during July and up to the end of August, 1937. About September 1, 1937, it was noted that he ate sticks and other foreign objects, and was less active than normal. Two weeks later there was considerable loss of weight and swelling of the abdomen. During the latter half of September there was hoarseness and a high pitched voice thought to be due to bronchitis. The dog continued to lose weight and the abdomen increased gradually in size. About December 1, abdominal paracentesis was performed and approximately 2 liters of blood-tinged fluid was obtained. The abdominal fluid promptly recurred and the hind legs became swollen. A second abdominal paracentesis performed about December 18, again yielded about 2 liters of blood-tinged fluid which contained flecks of fibrin. During the first week of January, 1938, the dog was observed by a local veterinarian in his animal hospital and a diagnosis of cirrhosis of the liver with ascites was made. The dog continued to eat well but the progress of the disease continued and, on January 14 an exploratory laparotomy was performed by a local surgeon, a friend of the owner. During this operation a large quantity of blood-tinged fluid was removed. The liver was seen to be greatly enlarged. This organ had rounded edges and its surfaces were brownish-red in color and slightly granular due to a thin deposit of fibrin. A small piece of the liver was removed and microscopic sections of it were later examined by Dr. E. W. Goodpasture, who found yeast-like parasites in Kupffer cells and in macrophages in lesions involving the portal areas. The intra-cellular parasite was tentatively regarded as a fungus, probably *Histoplasma capsulatum.*

Blood drawn from the dog on the day before he was sacrificed for autopsy was cultured in infusion broth (pH 7.2) and on potato-dextrose agar (pH 6.5). After incubation at room temperature (25—28° C.) for 16 days, a growth which appeared as minute, grayish granules was present in the infusion broth. This growth proved to be mycelial in character which indicated that it was a fungus. After 17 days incubation at room temperature the potato-dextrose agar plates were covered by small, white, cotton-like tufts of mycelium.

Ascitic fluid, urine obtained through a seared surface of the bladder, liver, and spleen tissue obtained at the autopsy were cultured at room temperature on the above-mentioned media. Cultures of these materials were also made on 10 per cent rabbit blood agar and on N. N. N. medium and incubated at 37° C. The urine cultures proved sterile. Growth became apparent in all the other cultures on the 13th day following the inoculations of the media. In all the cultures the growth was mycelial in character and yeast-like forms of the fungus were not observed.

The pathological findings in both the natural and experimental diseases induced by this fungus are strikingly similar to those reported as occurring in *Histoplasmosis* of Darling in man. The cultural characteristics of this fungus, in so far as has been determined by this study, also appear identical with those of *Histoplasma capsulatum*, the fungus which was first isolated from a case of *Histoplasmosis* of Darling in an infant by this writer and proven by him to be the cause of the disease.

Since its isolation the original strain of *Histoplasma capsulatum* has been studied in a number of laboratories and conflicting opinions have arisen regarding its classification. REDAELLI and CIFERRI, following their studies of this fungus, created the family *Histoplasmaceae* and included it with the families *Nectaromycetaceae Cif. and Red.* and *Torulopsidaceae Cif.* in the super-family *Adelosaccharomycetaceae Guilliermond,* comprising all the non-spore-bearing yeasts. Basing their evidence mainly on the pathology of the host, and the appearance of the fungus in the tissues, they placed *Cryptococcus muris* Shortt, 1932, in the genus *Histoplasma*, with *H. capsulatum* as the type species. MOORE, not in agreement with this classification, after noting endospores within the tuberculated spores which occur on the aerial hyphae, placed this fungus in the genus *Posadasia* which was created by Canton in 1898. He thus renamed this fungus *Posadasia* capsulata and regards the genus as an entity in the family *Coccidioideaceae,* which he in turn regards as closely related to the family *Endomycetaceae.* Dodge also places *Histoplasma capsulatum* with the *Coccidioideaceae* but retains the genus *Histoplasma* of which he considers this fungus the species type.

In spite of numerous attempts, DE MONBREUN never observed endospores within the tuberculated spores of *Histoplasma capsulatum.* He is convinced, however, that these structures function as asci because multiple germ tubes, often as many as 6, have been repeatedly observed growing from within them when they are placed on suitable media.

In order to detect possible cases of histoplasmosis that may otherwise be unrecognized, it is suggested that in all cases of obscure splenomegaly and lymphadenopathy smears of peripheral blood should be stained with WRIGHT'S stain and examined for the presence of the intracellular parasites. The parasites should also be searched for in biopsies of lymph nodes. Also, cultures of blood and lymph nodes should be made on such media as infusion broth and SABOURAUD'S agar or potato-dextrose agar (pH 6.5) and observed for at least three weeks. The characteristic tuberculated asci of *Histoplasma capsulatum* which develop on the aerial hyphae of the mycelium on solid media are easily recognized. To confirm the identity of the fungus cultures of it may be injected intraperitoneally into mice. The yeast-like intracellular parasites may usually be recognized in smears of the peritoneal exudate, and in sections of the infiltrated omentum of the infected mouse as early as 3 to 10 days following inoculation.

SHAFFER et al. (1939) reported on *Histoplasmosis* of *Darling.*

Histoplasmosis of Darling (Darling's disease) is a rare disease entity. As far as can be ascertained, until 1934 only seven cases had been reported in the medical literature, all of which had occurred in the Western hemisphere. Four were found in the tropics; three in Panama and one, in which the diagnosis was histoplasmosis of the lung, in Honduras. The remaining three were reported from Minnesota, California and Tennessee, respectively. The racial distribution was varied. Of the four patients in the tropics, two were Martinique Negroes, one a Chinese and the fourth a "native" of Honduras. The patients in the United States were a mulatto and two members of the white race, one of them an infant, none of whom had ever been in the tropics or had had any contact with tropical areas. Only in the case reported by DODD and TOMPKINS was the diagnosis made during life by the discovery of the parasite in supravitally stained blood smears. The organism was also successfully cultured from the tissues post-mortem.

In a case of splenomegaly and hepatomegaly with anemia in an American born girl aged 11 months, the history, clinical picture and course, as well as the post-mortem examination, supported the diagnosis of *Histoplasmosis* of Darling. It is believed that this is the fourth case to be reported in the United States and the eighth in medical literature.

AMOLSCH and WAX (1939) reported on *Histoplasmosis* in infancy. A fatal case of infantile histoplasmosis is reported. Apparently this is the 10th case of human histoplasmosis to be recorded, and the 2nd case observed in an infant.

The clinical manifestations were chronic paroxysmal cough, anemia, leukopenia, continuous fever, weakness and hepatosplenomegaly.

Of the organs, only the surgically removed spleen was available for study. This showed a marked proliferation of reticuloendothelial cells, many of which contained the fungus.

Phagocytic cells laden with parasites were found in blood smears from the circulating blood.

The *Histoplasma* is a fungus, the pathogenic form of which resembles yeast. The saprophytic form is a myceliate spore-bearing fungus whose exact taxonomic position is still in question.

Knowledge regarding the epidemiology and pathogenesis of histoplasmosis is incomplete.

MELENEY (1940) surveyed the publications on *Histoplasmosis* (reticuloendothelial cytomycosis).

Thirty-two cases of histoplasmosis, thirteen of which have not yet appeared in the literature, are reviewed. The spontaneous infection in animals is also noted. The disease is widely distributed throughout the world and will undoubtedly be recognized with increasing frequency in the future. Diagnosis and the possibilities of successful treatment are discussed.

NEGRONI'S (1940) extensive study on the first case of *Histoplasmosis* found in South America deserves special interest.

A fungus classified as *Histoplasma capsulatum* Darling, 1906, has been isolated from ulcers of a patient who died 3 months later.

This was the first South American case of histoplasmosis, the third case diagnosed when the patient was still alive, the third from which culture was obtained and the second case with cutaneous lesions (ulcers).

The chief mycological characteristics are the following:

Hyphomycetic culture (type III of Ciferri and Redaelli). Macro-morphological characteristics: On rich solid media: velvety or cottony, dirty white, zonate or hemizonate development. Reverse: uncolored, yellowish or brownish, zonate or wrinkled.

On CZAPEK medium and on potato-agar: poor, velvety or arachnoid development, of dirty white color and with an abundant submerged muycelium.

In liquid media: velvety pellicle.

Micro-morphological characteristics: hyalin, branched and septated mycelium of about 2μ in diameter. Racket mycelium and chlamydospores are present.

This fungus forms globose or pyriform, smooth or rough and thinwalled conidia of $1{,}87\mu$ to $4{,}67\mu$ in diameter.

Smooth or variously sculptured hypnospores or stalagmospores (papillate, verrucose or aculcate) are also produced. They are globose or pyriform, thickwalled and pigmented in old cultures, from 7,48μ up to 33,66μ in diameter.

The articles of vegetative mycelium have one or many nuclei; conidia and young hypnospores have only one. Nuclear division in vegetative mycelium is accomplished by amitosis and when a conidium is in formation it receives a prolongation of a nucleus of the mycelium and the nuclear division is also performed by amitosis.

Physiological characteristics: Optimum temperature for growth: 25—30° C. The following carbohydrates are neither acidified nor fermented: dextrose, galactose, levoluse, mannose, lactose, maltose, succhrose, raffinose, inuline and starch. Dextrose, galactose, mannose, maltose and raffinose are better assimilated than the other carbon source. Ethyl-alcohol, practically not assimilated. Cellulose and sculin: not attacked. Starch: not hydrolised.

It assimilates better pepton and asparagine than NO_3K and SO_4 $(NH_4)_2$.

Reduction of nitrates to nitrites: positive. SH_2 production: negative.

Indol and acetil-methyl-carbinol: not formed. Plain or litmus-milk: not modified. Calf-fat attacked. Gelatin: slowly liquefied.

The culture is killed after heating during 15 minutes in water-bath at 55° and by the action of formalin solutions of 1 to 2/1.000 during 24 hrs.

Yeastlike culture (type I). For the first time the transformation in vitro of the hyphomycetic culture into a yeastlike one has been obtained by the action on the first type of culture of saline solution, sodium hydrate solutions from 1/2.000 to 1/8.000 and more constantly with sodium borate solutions from 0,5 to 2/100. After a contact of 24 hrs. in these solutions the material was sown on blood-agar and incubated at 37°. It is believed that blastospores formation under these conditions depends on staling effects.

Macro-morphological characteristics: Colonies on blood-agar begin to appear after 3 to 4 days of incubation at 37° C. They are hemispheric, raised, moisted, shiny of 1 mm. in diameter more or less and of a creamy consistency. The development is confluent after a week.

On dextrose glycerin-agar: granular, thick, white, moist and brilliant development.

In liquid media: sediment without turbidity.

Micro-morphological characteristics: oval or elliptical blastospores from 2,30 × 1,53μ up to 7,65μ × 4,59μ (being the average of 4,59μ × 3,06μ) budding by one or two poles.

Uninucleate cells with direct nuclear division (amitosis) during sprouting.

Physiological characteristics: Optimum temperature for growth: 37° C.

Yeastlike culture requires complex proteids for its development. At room temperature and on common media it turns soon again to the hyphomycetic type of growth.

Intermediate culture (type II). In the first culture on blood-agar of the above mentioned experiment performed to obtain the yeastlike type of growth appeared cerebriform or umbilicated, dry (not velvety) and firm colonies of about 2 mm. in diameter.

Microscopic examination: filamentous mycelium, abundant chlamydospores, proteiform cells budding by one ar mony poles, moniliform filaments and typical blastospores.

Pathogenic power: laboratory animals and monkeys are sensible to the inoculation of either hyphomycetic or yeastlike types of cultures.

Testicular inoculation in guinea pigs has shown particularly severe.

CONANT (1941) made a cultural study of the lifecycle of *Histoplasma capsulatum Darling* 1906. 1. A. strain of *Histoplasma capsulatum* from a fatal case of histoplasmosis in a three-months old infant is studied. 2. The saprophytic filamentous form developed on SABOURAUD's glucose agar at room temperature is characterized by large tuberculate chlamydospores, not asci, as proven by appropriate staining methods. 3. The filamentous form is converted to the yeast-like tissue form without the use of animal inoculation by cultivation on sealed blood agar slants at 37° C. 4. From a cultural study of the complete lifecycle of *Histoplasma capsulatum* it is shown that the fungus should be placed in the *Moniliaceae* of the *Fungi Imperfecti.*

DE ALMEIDA and DA SILVA LACAZ (1941) described two cases of *Histoplasmosis* in Sao Paulo (Brazil). In one case the histoplasmosis was associated with chromoblastomycosis. In the other instance, *Histoplasma capsulatum* was isolated from the sputum of a patient, who suffered also from tuberculosis. They succeeded in transforming the cottony culture of this fungus into the yeast-like form by culturing it on serum-agar at room temperature.

MORRIS MOORE (1941) presented a valuable study on *Histoplasma capsulatum:* its cultivation on the choriallantoic membrane of the developing chick and resultant lesions.

It was hoped, that the successful inoculation of the chorio-allantois with *H. capsulatum* might accomplish a two-fold purpose. The first was to determine whether this fungus would revert to a parasitic morphology and produce a definite histologic picture in a short time as do many of the fungi; and secondly, to determine whether such inoculations would shed light on the character and determination of the various morphologic entities of *H. capsulatum.*

The technique of fertile egg inoculation as used here is essentially similar to that of GOODPASTURE and BUDDINGH. The cover-slip method was found best for watching the development of lesions on the chorio-allantoic membrane.

Fertile eggs were incubated 12 days in an electrical, thermostat-controlled incubator regulated to maintain a temperature of 98° F. The eggs were turned twice daily. A cm. square window was cut in the shell above the embryo, exposing the chorio-allantoic membrane. The membrane was inoculated directly with the fungus and the window was covered with a sterile cover-slip and sealed with a paraffin-vaseline mixture (9 parts vaseline, 1 part paraffin). After inoculation, the eggs were set in a bacteriologic incubator and maintained at a temperature of approximately 33° C., without turning. The membrane was watched daily through the window and 6 days later, when the patch on the membrane had shown marked change, the shell was cut below the window and the membrane exposed. The chorio-allantois was cut with a pair of fine, curved-end scissors, removed, fixed in Zenker's solution (with 5 per cent glacial acetic). After washing, dehydrating, clearing in xylol, and embedding in paraffin, it was sectioned and stained. Some of the sections were colored with hematoxylin and eosin to study the various types of membranal cells while others were stained with Loeffler's methylene blue and eosin in order to get a clear picture of the fungi.

The culture of *H. capsulatum* used in the inoculations was a 20-day-old sub-culture which showed a sufficient amount of growth of aerial mycelium with the various cells and morphologic structures characteristic of the fungus in culture. This strain of *H. capsulatum* was the one isolated by DE MONBREUN in 1933. In inoculating the eggs, care was taken to include only the fungus and none of the substratum.

No attempt was made at this time to determine whether the organism had spread into the body of the embryo. Attention was focussed only on the membrane, consequently the chick embryos were not autopsied.

Eggs incubated 12 days and observed 6 days after inoculation show: Macroscopically the lesion on the chorioallantoic membrane is a thickened, yellowish-gray plaque with some opacity in the region surrounding the somewhat raised patch. Scattered over the membrane are thickened regions showing a grayish infiltrate. These apparently are areas where the organism has been able to continue its growth and invade the chorio-allantois. The embryos were alive in most of the eggs although they were apparently considerably weakened.

Microscopically, a section through the plaque and the chorio-allantois shows a marked reaction both in the inoculum of the fungus and in the membrane. The ectodern shows marked proliferation in the region where the fungus has come in close contact with it. This is demonstrated by a definite thickening in some cases with elongation of the ectodermal papillae, in others the ectoderm seems to have lost its morphologic identity, resembling somewhat a "traumatic ulcer." There is a large accumulation of monocytes, some leucocytes and some eosinophil-like cells in that part of the ectoderm adjacent to the fungous inoculum. The effect of the infectious process is quite noticeable also in the papillae and in parts of the ectoderm where the organism has been able to invade, in the presence of leucocytes, monocytes and red blood cells. In these areas also can be seen a few isolated cells of *H. capsulatum*, as small spherical structures.

The mesoderm likewise shows an intense reaction at the sites of marked ectodermal response. In the area of the mesoderm immediately adjacent to sites of ectodermal reaction there is an increased infiltrate composed of leucocytes, fibroblasts and eosinophil-like cells. In some areas the fibroblasts seem to have invaded most of the mesoderm and the rapidity of the formation and spread of these cells is evidenced by the presence of mitotic figures in some cases. Here too are seen numerous eosinophil-like cells, free in the tissue or in the increased number of capillaries packed with red blood cells, some monocytes and the eosinophil-like cells. Just what part these eosinophil-like cells play in the reaction has not been determined. There can also be seen ectodermal pearls extending into the mesoderm as well as whorls of ectodermal cells and leucocytes and also a few giant cells of the LANGHANS' type. The leucocytes seem numerous in areas adjacent to the involved ectoderm, but decrease in number deeper in the mesoderm. In general, the mesoderm shows the most pronounced reaction at the region of marked ectodermal response, with a lesser mesodermal response in the areas of less proliferation of the ectoderm.

The entoderm likewise shows an increased thickening and reaction in the regions underlying the areas of marked mesodermal and ectodermal reaction.

On both the ectodermal and some areas of the entodermal surfaces, one is struck by the rather large number of red blood cells and monocytes. There is also noted a granular, pink-staining (eosin) exudate within which may be seen dispersed red blood cells, monocytes, nuclear fragments of degenerated cells, in some areas pink-staining granules, nests of monocytes and red blood cells, particularly on the ectodermal surface, in the base of the fungous inoculum adjacent to the ectoderm. At the base of the inoculum also, adjoining the ectoderm, occur filaments of *H. capsulatum* with some small spherical to ovoid cells of the fungus. In this area also there is an occasional nest of these parasitic cells surrounded by monocytes and red blood cells.

The growth and subsequent development of the fungus on the chorio-allantoic membrane is analogous to what has been described for other fungi grown on this membrane.

The development or conversion of the fungus from the saprophytic to the apparently parasitic stage is very interesting. As it is grown on an artificial substratum, *H. capsulatum* consists of filaments, conidia, chlamydospores, terminal hypnospores and tuberculate cells, stalagmospores or aleuriospores of some and referred to as ascus-like bodies in a previous publication. These structures can be seen on the freshly inoculated membrane. As the fungus becomes acclimated to the new medium the various cells begin to change their morphology. The filaments develop cross-walls which are formed so as to produce spherical to ovoid clear spaces in the hypha. These spaces tend to become somewhat enlarged as the transverse septum takes on a definite form and finally there is an indentation and pinching off along the cross-wall to form single cells. The process is analogous in all respects to the formation of arthrospores and then oidia in various yeastlike organisms as *Mycoderma, Geotrichum* and somewhat as seen in *Candida* (Monilia).

The cells become free and dispersed, measuring approximately 2 to 5 microns in diameter, depending on the size of the hypha or the cell from which they develop. However, these cells go through a process of budding resulting in smaller cells so that at the periphery of the inoculum, or in the center of the nests described previously, there are seen clusters of fungus cells measuring approximately 1.5 to 4 microns in diameter. This would appear to correspond to the type of cell seen in human parasitized tissue except that the capsule noted in human tissue is lacking in the peripheral organisms. This can be readily understood since the capsule is a tissue defense mechanism and the fungi in this area are not directly associated with the tissue. By examination of the areas where the cells are in contact with the membranous tissue, a capsule can be made out distinctly on the small yeastlike cells. Another important feature, revealed by a close examination of these yeastlike organisms, is that they lack a definite nucleus, but have instead a chromatin network with metachromatic granules. A section through a cell, therefore, makes it appear as though there is a corona of granules on the inner surface of the organism with a polar concentration. This feature has been observed in cells in human tissue and has been commented on by numerous workers.

The fate of the tuberculate cell (stalagmospore, aleuriospore or ascus-like cell) is of great interest particularly since the nature of this structure has been under dispute. From the information gathered as a result of these inoculations, the probable ascus-like nature of these cells is indicated since they become converted into numerous yeastlike cells. These are apparently not definite in number, but can be seen in clusters terminally or laterally on a filament. The cell first becomes contracted and then takes on the definite form of a rosette or mulberry-like cluster and finally a group of organisms is formed. As far as can be determined, the cell wall becomes disintegrated during the process as is evidenced by the pink-staining material, while the yeastlike cells are taking form.

RHODES' et al. (1941) report on *Histoplasmosis* is also of interest.

Interest in the fungus disease histoplasmosis, described by DARLING several years ago, has been revived recently because of the number of new cases reported within the past two years. In the Mississippi Valley region alone, nine cases have occurred within a year, and HUMPHREY would not attribute the increase to better diagnosis alone, but to an increased incidence of the disease as well. At the present time, MELENEY has shown in his review of thirty-two cases, only ten of which were reported prior to 1939, that histoplasmosis can no longer be considered rare, but must be ruled out in any condition characterized by an enlarged spleen and liver, enlarged lymph nodes, leucopenia, anemia, or chronic respiratory infection.

Four cases of this disease in infancy, characterized by extreme emaciation, splenomegaly, leucopenia, anemia, and pyrexia, have been reported. It was reported for the first time by DODD and TOMPKINS in 1934. This was also the first case to be diagnosed before death and the first from which a culture of *Histoplasma capsulatum* was recovered. Later, in 1939, cases were reported in infants by AGRESS and GRAY, SHAFFER, SHAUL, and MITCHELL, and AMOLSCH and WAX. The present case was that of a 3-month-old infant, apperantly the youngest to be recorded, with a culture of *Histoplasma capsulatum* obtained from the blood stream twenty-four hours before death.

Blood cultures taken the day before death were later found positive for a fungus identified as *Histoplasma capsulatum*. This fungus grew as a typical mold on SABOURAUD's media at room temperature. It was at first cottony white, but became brownish in older cultures. Microscopically the fungus filaments supported large warty chlamydospores which were characteristic of *Histoplasma capsulatum*. On blood agar at incubator temperature the fungus developed as a yeastlike growth. In these cultures, the fungus appeared as small, budding, yeastlike cells typical of the parasitic stage found in infected tissue.

KEY and LARGE (1942) reported on *Histoplasmosis* of the knee.

In so far as the authors have been able to determine, none of the cases in the literature presented any involvement of the joints or muscles. Recently one of the authors has had under his care a patient from whom the sections from the specimen of the knee joint showed the typical *Histoplasma capsulatum* of Darling. The case is being reported because it is believed to be the first instance in which the disease has been known to involve a joint, and also because the case closely resembled tuberculosis of the knee joint, and was so diagnosed until it was found that the guinea-pig inoculation and the tuberculin test on the patient were negative. It was then thought to be a low-grade infection of unknown origin. The limb was amputated and the correct diagnosis was arrived at only when it was made by Dr. MORRIS MOORE, of the Barnard Skin and Cancer Hospital, to whom the slides from the tissues were submitted. The case report follows:

A white male, aged forty-seven years, was admitted to the Medical Ward on May 14, 1941, with a history of aching pain and disability in the left knee of seven months' duration. The personal history revealed that the patient had been born in southeastern Missouri, and had lived there on a farm all of his life. The pain in the knee developed gradually, and was not preceded by any definite injury. The knee became swollen and stiff. The pain increased and was aggravated by movement.

Roentgenograms of the left knee and chest were taken. The lateral view of the knee joint showed thickening of the soft tissues and moderate bone atrophy with spotty atrophy of the bone structure. The anteroposterior view showed spotty atrophy and no decrease in the joint space, and in neither view was there any evidence of new bone formation. The chest film showed rather well-defined rounded areas of almost complete opacity, measuring seven by seven centimeters, in the lower half of each lung field between the fourth and sixth ribs anteriorly. Although these areas had an appearance suggestive of encapsulated interlobar collections of fluid, roentgenographic diagnosis was deferred.

Sections from three areas of the joint were studied: (1) the fibrous tissue of the synovial membrane; (2) the cellular layer of the synovial surface; and (3) the cortex of the femur beneath the synovial surface. Examination of these sections revealed the following:

1. The fibrous portion of the synovial surface showed relatively little reaction, and the dense fibrous tissue was, as a rule, not invaded by the inflammatory process, although a few monocytes which contained one or two organisms were seen beneath the surface, and there was a slight amount of proliferation of the surface cells.

2. The cellular portion of the synovial surface was markedly thickened, and was composed of a mass of mononuclear cells embedded in a youthful connective-tissue network. The blood vessels were moderately increased in size and number. The synovial membrane was transformed into a mass of granulation tissue, which was intensely infiltrated with macrophages and giant cells, and was characterized by the presence of numerous areas of focal necrosis which resembled the caseation found in tuberculosis. In these areas there were degenerating cells and numerous giant cells, some of which also were degenerating. The great majority of the cells in the inflammatory exudate were mononuclear phagocytes or monocytes. Many of the phagocytic cells contained minute spherical bodies which varied in size from three to five micra in diameter. These bodies possessed a clear cytoplasm which was surrounded by a refractile ring and contained a deeply staining central mass or nucleus which was usually spherical, but might be irregularly ovoid or semilunar in shape, and was about one-half the diameter of the parasite. These were the *histoplasma capsulatum* of Darling. In some areas the parasites were present in enormous numbers, and crowded the bodies of the large macrophages and giant cells. Where these cells were burst or degenerating, the parasites were free in the tissues or in the necrotic debris, but for the most part they lay in the cells. Relatively few of the monocytes contained any other phagocytized material, and very few of them contained red-blood cells. The endothelial cells or monocytes merged into the phagocytes, and these, in turn, merged into the giant cells which varied greatly in size; some of them contained fifty or more nuclei and resembled foreign-body giant cells. There was a striking absence of polymorphonuclear leukocytes in the diseased areas of the tissue.

The pathological picture resembled that of a tuberculous synovial membrane, except that there were no tubercles and the giant cells were of the foreign-body type. When the sections were stained for acid-fast bacilli, no organisms were seen.

The synovial surface was covered with an exudate which contained many well-preserved cells, mostly mononuclear phagocytes and small round cells (probably lymphocytes and plasma cells). It also contained a considerable number of giant cells, some of which were quite large, exhibiting thirty-five or more nuclei in a single five-micron section. In some areas the exudate was composed of a mass of fibrin and of necrotic cells. In the bodies of the mononuclear phagocytes and of the giant cells in the exudate, there were large numbers of the yeastlike organisms. Some of these organisms were free in the coagulated exudate, but, for the most part, they were intracellular.

3. The invasion of the bone was also similar to that which occurs in tuberculosis. The infected inflammatory tissue extended down through the nutrient canals of the cortex and spread out in the bone-marrow spaces. The trabeculae in the bone marrow tended to be eroded by the granulation tissue, and showed little or no tendency to the production of new bone. Likewise, the bone was not killed by the juxtaposition of the infected granulation tissue. Outside of the areas where the bone marrow had been invaded through the nutrient canals, the cancellous bone and marrow appeared to be normal and no organisms were found in this tissue. They were, however, quite numerous in the cells of the

granulation tissue which had invaded the bone marrow, but were not so numerous as they were in the inflammatory tissue of the cellular portion of the synovial surface.

L. Rhinosporidiosis

GRIFFEY (1939) reported a case of *Rhinosporidiosis* from Texas.

E. K., a normally developed white boy, aged 10 years, presented himself on December 14, 1938, with a small strawberry-colored mass attached to the caruncle of the right eye.The child lived on a farm and helped take care of horses and cows. There are several children in the family but no others are affected.

The mass was nodular and solid, encapsulated, roughly pear-shaped, 12 by 10 by 6 mm. in size, and was attached to the upper edge of the caruncle and scleral conjunctiva by a thin stalk 5 to 8 mm. long. The mass was lying partly in the palpebral fissure so that the lids could not be completely closed at the inner canthus.

The mass was excised at the base of the pedicle; one suture was required to close the wound. The patient was last seen on January 14, 1939, and showed no sign of recurrence.

The mass presents a chronic inflammatory reaction around spores that are numerous in the subepithelial stroma and that are also present in the surface epithelium. The spores are so numerous that several low-power fields show as many as 40 encapsulated organisms that vary in size from about 30 to 200 microns in diameter. With hematoxylineosin staining, most of the organisms show a thick amphophilic capsule surrounding a foamy granular material whose center contains an ovumlike structure with a distinct limiting membrane, a pseudogranular protoplasm, and a slight eccentrically placed rounded basophilic body about one third the diameter of the ovumlike structure. The larger organisms contain hundreds of blue-stained endospores filling the entire interior. The capsules of the large, mature organisms are thicker and somewhat refractile. The capsules stain blue with the Masson stain, and with Giemsa stain they stain red except in the largest ones, which stain blue. Within and around a recently ruptured organism there are infiltrating leucocytes. Each escaping endospore would seem to begin a new cycle of enlargement with multiple endospore formation.

The chronic inflammatory tissue is characterized by diffuse edema and an exudate composed chiefly of plasma cells mixed with lymphocytes, polymorphonuclear leucocytes and a few large mononuclears. Around the medium-sized and small encapsulated spores cellular reaction is not intensified, but since the cells are more numerous around groups of small forms it would seem that the rupture of mature organisms incites the cellular activity. The tissue is quite, vascular. The vascular endothelium is not particularly swollen. The 1 mm. dark cyst appears over an ulcerated area and contains one very large body filled with endospores, a recently ruptured body, and numerous free encapsulated endospores, some as small as 15 microns in diameter and some of which show on the surface. Infiltrating leucocytes and necrotic tissue are also present here. The squamous epithelium is greatly thinned out over much of the surface but in places it is thickened and infiltrated with leucocytes.

RUCHMAN (1939) described the first occurrence of *Rhinosporidiosis* (SEEBER) in a female in North America.

ASHWORTH furnished the first satisfactory description of *Rhinosporidium seeberi*. It was first mentioned by SEEBER, of Argentina, in 1900, and it was independently discovered in India, where the first case of involvement was reported by O'KINEALY. MINCHIN and FANTHAM studied O'KINEALY's material and created the name *Rhinosporidium kinealyi,* which remained in vogue until ASHWORTH demonstrated SEEBER's priority and amended the name to its present form *Rhinosporidium seeberi.*

To date there have been 53 cases reported, from the following locations: 4 from Ceylon, 40 from India, 5 from North America, 3 from South America and 1 from Italy. In 4 of the 53 cases the disease occurred in females. It usually attacks young subjects — in most of the reported cases the patients were under 30.

In 40 of the 53 cases the lesion was in the nose, mainly in the anterior part. The conjunctiva was affected in 8 cases. The ear also has been involved. BEATTIE, professor of pathology at the University of Sheffield, England, was the first to describe the parasite in the ear. He devised a staining method for this parasite, which is used to this day.

The staining method in as follows:

1. Stain by RICHARD MURS alcoholic eosin method, omitting methylene blue.
2. Place in a 2 per cent iron alum solution for fifteen minutes.
3. Transfer to a 0.5 per cent watery solution of hematoxylin for fifteen minutes.
4. Wash and decolorize carefully after the manner of Heidenhain.
5. Stain for about one minute with 0.5 per cent alcoholic solution of Lichtgrun (Gruhber) and mount in Canada balsam after dehydrating and clearing.

The author's case is the fifty-sixth to be reported, the eight case reported in North America and the first on record in North America in which the condition occurred in a female.

The patient was 33 years of age and married; she had 5 children. She was born in Galicia, then a part of Austria.

She had come to the United States in 1922, at the age of 16, and got a job in a window shade factory.

For many years she had apparently enjoyed good health. She was never troubled with head colds, sinus disease or infections of the upper part of the respiratory tract until two and a half years before she came to the author's attention, when the current trouble began. She claimed that she had caught cold and the left side of her nose became stuffed. This nasal blocking became worse with time, but since she had no other symptoms she did not seek medical aid.

The right side, except for a slight mucous discharge, appeared normal. The left side showed a partial closure between the septal wall and the inferior turbinate body. At first it appeared like a septal-turbinal synechia, but after application of 10 per cent cocaine and epinephrine hydrochloride the inferior turbinate shrank and left the septal wall. Attached to the lower anterior part of the septum was an elevated growth the size of a pea, leaflike, and with well defined points radiating in all directions. The surface was smooth and the color pale red. On slight manipulation the growth began to bleed. A mucopurulent discharge stained with fresh blood was spread over the inferior turbinate and the floor of the nose.

Repeated operations for the removal of the growth and its adjacent normal tissue were necessary. The treatment may be supplemented by the use of chlorhydroxyquinoline ointment and of a 5 per cent aqueous solution of antimony and potassium tartrate.

Another case of *Rhinosporidiosis* of the *conjunctiva* was presented by BARNSHAW and READ (1940).

A youth of 17 years, reported to the ophthalmic clinic of Dr. J. S. SHIPMAN at the Cooper Hospital in Camden, N. J., on May 19, 1939. He complained of a growth beneath the right upper lid, which had been present since March 1939. Dr. LOUIS RUTTENBERG examined the patient and, on eversion of the upper lid, found a quite vascular papillomatous growth springing from the palpebral conjunctiva at the upper border of the tarsal plate. The lesion measured 2 by 4 mm.

The chief interest lies in the organism itself, which occurs free in the connective tissue and at first measures about 6 microns in diameter. This is called the trophic stage. It has a dark cellular membrane, the chitinoid envelop and a nucleus with a karyosome. The cell enlarges to 50 to 60 microns when the nucleus begins mitotic division. At about 100 microns the parasite begins to develop a layer of cellulose-like material on the inner surface of the envelop. At about 140 microns the parasite contains about 16,000 nuclei; as they have now developed into definite cell entities they are in reality young spores, and the whole parasite is termed a sporangium. About two thirds of the spores develop and on enlarging acquire refractile spherules in the cytoplasm. The sporangium becomes 250 microns or more in size, and at this point the wall is stretched thin until finally the envelop is broken and the young spores are discharged. These have already reached the trophic stage and are ready to develop into mature sporangia in turn.

In the sections parasites of 20 microns or less are seen, and the largest sporangium measures about 180 to 200 microns. In the latter, small spores with refractile spherules are seen. The wide cellulose shell is striking. In the smaller organisms the chitinoid envelop is more easily demonstrated.

ANDERSON and BYRNES (1939) described a case of *Rhinosporidium* of the *conjunctiva*.

Rhinosporidiosis is a disease of the mucous membranes characterized by the formation of peculiar and distinctive polypi. Formation of these polypi is excited by the invasion of the mucous membranes by an organism that has been studied by ASHWORTH and assigned to the lower fungi (*Phycomycetes*), suborder *Chytridinae*, provisionally placed near the *Olpidiaceae*. This particular fungus was originally isolated from a nasal polyp by GULLERMO SEEVER in Buenos Aires in 1896. It was rediscovered by Major O'KINEALY of the Indian Medical Service in 1903. The disease has been described as occurring in man, cattle, and horses. It has been reported from Asia, the Philippine Islands, North and South America, from Africa, and from Europe.

The first conjunctival polyp known to be due to the *Rhinosporidium* was observed and reported by KIRKPATRICK at Madras in 1909. ELLIOT and INGRAM reported on a conjunctival form in 1912; TIRUMURTI in 1915; WRIGHT in 1922; DUGGAN in 1928; KURUP in 1931; RAO in 1931; and KAYE in 1938. KAYE reported from South Africa; all other reports were from India. Recently reports of nasal polypi due to this organism have been reported in Texas. A total of seven cases of nasal polypi due to the *Rhinosporidium* have been reported in the United States according to CALDWELL and ROBERTS.

The infection observed, occurred in a 12-year-old Negro boy, who presented himself in February, 1938, with a history that for the past 12 months he had noticed a growth on the lower lid of his left eye which, while growing slowly, had now reached the point at which it interfered with closure of the lid. The growth was first observed by the patient in December, 1937. There was no history of previous trauma nor of any similar infection or growth in any of his immediate associates. During the first six years of his life he had lived on a farm in the eastern part of North

Carolina, but for the past six years had lived in the city of Durham. He had not been associated in any way with pets, horses, cattle, or agricultural pursuits.

Examination revealed an irregularly lobulated, pinkish-red growth extending or protruding from beneath the lower lid near the external canthus. This growth was definitely pedunculated and attached in the fornix by a broad base. It measured roughly 8 by 4 by 2 mm. There was some lacrimation but no other evidence of irritation. On casual examination it resembled very much the fungoid granulation which one sometimes sees following the curettement of chalazia, and it was first presumed that such was the nature of this lesion.

The cases reported by the Indian observers and the one here reported have the following characteristics in common. The duration of the disease was usually about one year. The lesion was painless, the patient coming in for relief of mechanical obstruction to lid closure. The preauricular gland was not enlarged. The conjunctival polyp averaged in the fresh state 8 by 4 by 4 mm. In the nose the polyp was said to have long, fingerlike processes having semitransparent edges and a band of fibrous tissue running down the middle like the midrib of a leaf. In the conjunctiva the picture was a modification of the nasal form. It was described by one author as having the appearance of a discolored strawberry. Another compared it to a gelatinous "cockscomb." Dilated tortuous conjunctival vessels were observed coursing over the mass in all cases.

M. Sporotrichosis

WADE and MATTHEWS (1938) reported on bilateral *Sporotrichosis* of the breast.

Female, 20 years of age, presented herself with the chief complaint of "trouble with the breast." She stated that one and one-half years previously she was kicked in the left breast by an infant with whom she was playing. The trauma was trivial, and she forgot about it until about four months later, when she noted a small lump in the breast at the site of the bruise.

This case was interesting because of the chronicity of the condition and the apparent difficulty of making a diagnosis. Incision and drainage, presumably for abscesses, had been done on two occasions, with uniformly poor results. The general health of the patient had been unaffected. The complication of a five months pregnancy made it imperative to establish a definite diagnosis quickly in order to prepare the breasts for parturition.

In the differential diagnosis we considered tuberculosis, syphilis, actinomycosis, aspergillosis, other rarer types of fungous or yeast infection and lastly a streptococcic or staphylococcic infection. Tuberculosis was excluded by the normal roentgenographic appearance of the chest and the absence of other clinical signs of tuberculosis of the skin. The primary lesion of syphilis was excluded by the dark field and serologic studies. The tertiary gummatous form of syphilis likewise was excluded by the negative reactions to the Wassermann and Kahn tests and by the absence of other stigmas of either congenital or acquired syphilis. The exclusion of the various fungi and yeasts was a laboratory problem, and in this case they both found the causative organism in smears and at biopsy and were able to culture it. Streptococcic and staphylococcic infection were ruled out by laboratory methods. Finally, as an aid in the differential diagnosis, the response to iodides was rather spectacular in this type of infection, although in certain cases of blastomycosis, therapeutic response to iodide medication is rather marked.

After the diagnosis had been definitely established, oral therapy was begun with the use of potassium iodide, starting with 0.3 cc. three times a day and increasing to 2.5 cc. three times a day. At first, potassium iodide in 1 Gm. strength was administered intravenously every third day. Local application of a weak tincture of iodine was employed on the open lesion. By the end of the second week the patient had not exhibited any sign of overdosage of iodide (in these cases the patients seem to tolerate iodides exceptionally well), and the lesion in the right breast had almost completely healed. At seven months the patient was delivered of a normal healthy infant. The breasts were completely healed, but it was thought wise to give the infant a formula immediately as further iodide therapy was necessary to prevent recurrence of symptoms in the patient. It is wise in these cases to continue treatment for at least two months after all open lesions are apparently healed.

Sporotrichosis is also a rarity in Argentina and in Brazil. MAZZA and SCHREIBER (1939) reported the first typical case of this fungus disease in the North of Santa Fe. RABELLO Jr. (1938) reported one case from Brazil.

NEGRONI (1940) made interesting observation of the transformation of *Rhinocladium* (Sporotrichum) Schencki in vitro into a levuriform culture.

He has obtained sprouting cell formation from filamentous cultures of *Rh. schencki* by the action of saline solution or better still of 1 or 2 % sodium borate solution during 24 hs. at 37° C.

The yeast-like cells produce pasty colonies and keep indefinitely this characteristic provided that transfers are made on blood-agar and incubated at 37°.

This transformation is reversible. At room temperature the filamentous type of culture develops again.

The yeast-like type of culture of *Rh. schencki* loses its fermentative and elective (auxanogramm) activities on sugars. The nitrogen auxanogramm is positive only for peptone. This culture keeps its pathogenicity for rats. Seeding material from the experimental lesions on blood-agar and incubating it at 37° we have obtained the yeast-like type of culture, while on wort-agar and at room temperature the filamentous type has developed. The same results were obtained when material from the lesions produced by the inoculation of the filamentous type of culture of Rh. schencki was seeded on the same media.

GASTINEAU, SPOLYAR and HAYNES (1941) reports on *Sporotrichosis* as an occupational infection in florists.

Since the description of the sporothrix by SCHENCK in 1898, 200 cases had been reported in the United States to 1937, according to GREENBURG, with 6 additional ones from 1937 to January 1940, making a total of 206. Study of the literature revealed that this series of 6 cases is the largest on record, and it becomes doubly interesting in that the epidemic was confined to florists. Of the 206 patients described in the literature, 3 were florists. Before this epidemic, 6 cases had been reported in Indiana since 1898.

That the disease is endemic in the Mississippi River basin was shown by FOERSTER'S study, in 1924, wherein he was able to allocate 130 cases to this area. Ten cases which have occurred since then can be definitely added to the number in the basin area. Sixty-seven per cent of the reported cases have occurred there.

Sporotrichosis is primarily an occupational disease of agricultural manual workers. Since the disease is one affecting agricultural manual workers and tends to be disabling and chronic if not diagnosed early, the worker undergoes an economic loss out of proportion to his disease; he may also suffer a stigma due to a wrong diagnosis. Prompt diagnosis and treatment are essential.

Trauma was noted in 80 of the reported cases. Five deaths have occurred. The age distribution in 112 cases was 18 to 50 years and in 27 cases 2 to 12 years. In an epidemic of sporotrichosis affecting 6 florists in central Indiana, 4 had received previous surgical treatment for primary lesions. All 6 had a history of trauma. Five had lesions of a lymphatic type, and 1 had generalized lesions. Two were husband and wife and were probably infected form a common source.

No other florists with sporotrichosis have been observed since this epidemic.

Industrial epidemiologic studies indicate that sphagnum moss may be the source of sporotrichosis among florists.

The identification of S. schencki in the 6 cases reported provides an example of the manner in which a governmental agency can assist physicians in private practice by doing epidemiologic studies for them.

Sporotrichosis is relatively rare in the South and Southeast of the United States. DAVIS (1940), in St. Petersburg, Florida, FRENCH (1941), Miama, Florida, reported typical cases of Sporotrichosis. FOGEL and MARTIN (1940) described a case of this infection in a 7-year-old white girl, fairly rare in children. In the latter case Tularemia was considered as a possible diagnosis, but the afebrile course, lack of systemic reaction, and negative agglutination against *P. tularensis* made it unlikely. Syphilis was eliminated because of negative dark-field preparations of the chancre and repeated negative Wassermann tests. Low-grade pyogenic infection would not respond so dramatically to iodides.

It is of importance to note that diagnosis was not possible in this case by microscopic sections or direct examination of material taken from the primary lesion, but was made only by culture of the biopsy material, with inoculation of several slants.

N. Maduromycosis

NEGRONI and TEY (1939) carried out mycological investigations in the first Argentine case of *Maduromycosis* with black granules.

They studied a strain of *Aspergillus* isolated in pure culture, at different opportunities, from the first Argentine case of black grain madura foot.

The grains were twisted and had 3 mm. of diameter, more or less. Its microscopical examination revealed a septated mycelium of 3μ of diameter numerous chlamydospores up to 20μ of diameter, enclosed in an amorphous and brown-colored intercellular substance.

This *Aspergillus* formed on solid media green velvety colonies with yellow zones. Under the microscope it showed a vegetative mycelium of 3,06—7,65μ of diameter and two types of fructifications.

a) Imperfect fructification:

Erect conidiosphores of 234 × 4,59—15,3μ with smooth walls. Globose or elongated conidial heads of 72—108μ of diameter, with loose chains of conidia. Globose or pyriforme vesicles of 12,24—30,6μ of diameter. Sterigmata in one series of 6,12—10, 71 × 3,50—3,28μ. Globose or elliptical and rough conidies of 4,59—6,12μ.

b) Perfect fructification:

Globose and yellowish perithecia of 55μ to 224μ of diameter. Globose eight spored asci of 11μ of diameter. Elliptical and rough ascospore with two equatorial crests limiting one furrow, of 4,59 × 3,83μ.

Physiological characteristics. — Optimum temperature for development: 30° C. Sugars: not fermented. Elective power for different carbohydrates: galactose, succhrose, maltose, dextrine, manitol and sorbitol +++, dectrose, mannose and levulose ++, lactose and inuline. The sign + signifies the comparative diameter of the colonies on synthetic media with different carbohydrates.

It grows in media containing 3 % of ethylic alcohol as the only source of carbon. Starch: not hydrolised and cellulose not attacked.

Assimilation of peptone and asparagine better than urea, NO_3K and SO_4 $(NH_4)_2$.

Gelatine: Slightly liquefied. It does not form indol, acetyl-methyl-carbinol and SH_2. Reduction of nitrates to nitrites: negative. Litmus milk: not modified. Bacteriolytic power (staphylococcus, B. pyocyaneus and prodigiosus): negative.

They have classified this fungus as *Aspergillus* (Eurotium) chevalieri Mangin, 1909.

DA FONSECA, and DE ARÊA LEÃO (1940) presented a contribution to the knowledge of the blastomycoid granulomatosis along with the causal organism of JORGE LOBO'S disease.

JORGE LOBO, from the Recife Medical School (Pernambuco, Brazil) described in 1933 a new type of human mycotic disease, then placed among the so-called blastomycosis, and which has been called "blastomycose, Jorge Lobo's type" and "Lobo's disease."

In LOBO'S papers information will be found about the clinical and histological features of his case, as well as a few data about the morphology of the fungus in the human lesions. The present publication, therefore, deals with the study of the parasite as it appears in artificial cultures.

As the causal organism of JORGE LOBO'S disease displays morphological and pathogenic characteristic which justify its place in the genus *Glenosporella* and as it is easily distinguishable from the known species of this genus, a new species is established for it: *Glenosporella Loboi*, n. sp.

"*Glenosporella Loboi*, O. da Fonseca et Arêa Leão, n. sp.

"Parasite of the skin and subcutaneous tissue of man, there appearing as roundish, hyalin cells, with double contoured cell-wall, isolated, geminated or irregularly grouped. Reproduction only by budding. When sphaeroidal, measuring 6.0—11.0, on the average 8.2 micra in diameter. When ellipsoid or ovoid, measuring 8.0—9.0 × 10.0—14.0 micra in diameter. In artificial culture, at room temperature, on all usual media, giving rise to white colonies covered with aerial hyphae, reaching after 3 weeks 1.0 — 1.5 centimeters in diameter. Mycelium filamentous, septate, branched, hyalin. Arthrospores always scarce, cylindroid or ellipsoid, hyalin, with 5.0 — 6.5 × 1.75 — 2.0 micra. Aleuroconidia numerous, continuous, sphaeroidal, ovoid or ellispsoid, hyalin, provided with double-contoured cell-wall, measuring 3.5—10.0 × 1.5—8.0, on the average 6.2 × 4.8, developed terminally on very long hyphae which reach 20.0 or more micra in length and 1.5—2.0 in breadth. These aleuroconidia are of slow and late formation and are not liberated until the mycelium where they are born is destroyed. Intercalary chlamydospores or chlamydoaleuria may sometimes be found.

Type-culture in the mycological collection of the Laboratory of Parasitology, National Faculty of Medicine, University of Brazil, under number 1488.

DE ALMEIDA and BARBOSA (1940) contributed to the knowledge of *Maduromycosis* (chromoblastomycosis) in Brazil.

They presented a synopsis of maduromycosis observed in Brazil. Data on 15 cases were obtained; etiological agents from 13 cases have been isolated and studied. The fungi were distributed as follows:

Genus *Madurella* — 4 cases, following species:

M. micetomi, *M. Ramiroi*, *M. Oswaldoi* and *Madurella sp.*

Genus Monosporium (Scedosporium), species apiospermum 4 cases.

Culture of one of these last cases is considered by Lobo and Leão to be *Acremoniella Lutzi.*

Genera *Aspergillus, Cephalosporium, Acremonium* and *Candida* are represented by one sample each, and genus *Indiella* presents two samples, according to the A. A.

From one case culture was not obtained.

Arêa Leão and Lobo (1940) described *Maduromycosis* with white grains caused by *Acremoniella Lutzi* n. sp.

The authors refer to the few cases published in the medical literature on human mycoses caused by some species of the genus *Acremoniella* Saccardo, 1878. In their own observation the disease started five years ago with formation of long chains of nodular lesions which broke down and formed fistulas. Thick pus with yellowish-white granules of variable size had been continuously discharged. The new species has the following characteristics: it easily grows on the usual culture mediums with colonies initially white producing abundant aerial hyphae. After 20 days at laboratory temperature it turns dark gray to dull black. This color is more intense and appears earlier in Czapek's medium. At the beginning, on the surface of the medium black dots appear which later invade the medium becoming nearly black. Here and there white dots are scattered in the black.

The microscopical features are as follows: Hyphae are white or slightly brown, septate, with smooth walls. Conidiophores are simple, lateral, 20 to 32 μ long and 1 to 2 μ in breadth. Conidiums are formed single, terminal. They are black with smooth wall, about 3 to 6 μ in diameter.

Dixon (1941) reported on *Sulfanilamide* therapy in *Madura foot.*

Fifteen years ago a colored school teacher, now fifty-five years old, used a pair of shoes that had been worn by a friend in Mississippi. (This is the only suggested source of infection). About two weeks later she developed a severe aching pain in the left great toe which was constant day and night. The dorsal part of the toe began to swell and soon broke down and began to drain. Ulcers appeared around the ankle, and draining sinuses developed on the side and plantar surface of the foot. She states that the drainage from these sinuses sometimes contained "little yellow balls." During the next seven years this colored woman visited numerous doctors, clinics, and hospitals, and, in spite of a negative Wassermann, was often treated for syphilis. In August, 1932, one toe, which had almost sloughed off, was amputated. A few months later when the disease had continued to progress, the left leg was amputated just below the knee. Unfortunately this specimen was not preserved, but was reported grossly to show "arteritis." Tragically for the patient, shortly after the amputation, the disease appeared in the other foot and ran the same course as in the original infection.

The patient had repeatedly refused surgical removal of the remaining foot. At this time the foot was enlarged and markedly deformed. It had a mottled appearance from pigmented areas mingled with scarred areas extending eight to ten inches above the ankle. There were small draining sinuses on the sides and plantar surface of the foot. Granules were not noticed in the discharge at the time. They could, however, be obtained by irrigating a sinus with normal saline. There were large superficial ulcers on both sides of the foot, and X-rays showed marked rarefaction of the bony structure.

After a year of unsuccessful local treatment, the foot was definitely worse. Since the causative organisms of mycetoma and actinomycosis are quite similar, this patient was started on eighty grains of sulfanilamide daily for four days, then sixty grains daily for four months. This she tolerated remarkably well. After the first four days the discharge had subsided and the pain had ceased. By the end of the third month healing was complete except for deformity. She was able to walk on the foot by wrapping it in several thicknesses of cloth.

Twice during the past eight months a small ulcer appeared on the foot which cleared up promptly with resumption of sulfanilamide. Apparently the lesions were reinfections, which is quite likely from the unsanitary little shanty where she lives. No other treatment was used with the sulfanilamide.

The significant fact is that a case of chronic draining sinuses of the feet, of fifteen years duration without a remission, has now been completely healed for eight months by sulfanilamide therapy. This case has had the clinical course, and physical characteristics of maduromycosis.

NINO (1941) reported a case of *Maduromycosis* with white grain, elicited by *Monosporium apiospermum* in Argentina. This observation was made in the State of *Neuquen* and it was the first one found in this country. The causative organism was identified as *Monosporium aspiospermum*, Saccardo, 1911.

ALMEIDA and BARBOSA (1940) described the *Cephalosporium Recifei* LEÃO and LOBO with characteristic features on CZAPEK'S medium. The fungus was obtained from a case of mycetoma in Pernambuco and it was the first case observed with this causative fungus.

O. Actinomycosis

The newer methods of *Therapy* of *Actinomycosis* were discussed by several authors.

SCHUCHARDT (1939) reported on *Vaccinotherapy* of Actinomycosis. Fourteen cases of progressive actinomycosis with proven bacteriologic findings have been treated by this method. Among these 14 cases there were nine moderately severe and five severe cases of the disease. They have been previously treated by X-ray irradiation for years without any success. The result of the specific vaccinotherapy was very favorable. Twelve patients were definitely cured, two others, at the same time, almost cured. The period of treatment was, in the average, from 2 to 3 months; in stubborn cases, up to six months. The results were equally good with both autogenous and heterogenous vaccine. The heterogenous vaccine has the advantages that one can avoid a waiting period of four to six weeks which is necessary for the preparation of the autovaccine, let alone the possibility that the culture fails due to technical difficulties. The dosage of the vaccine is of absolute importance and it can be handled only individually. The use of the vaccinotherapy requires a good deal of personal experience in immunotherapy. The cervicofacial actinomycosis should be treated, however, also in the future above all, with X-ray irradiation and iodine-iontophoresis, while the early application of the vaccinotherapy promises far the greatest success in cases of thoracic and abdominal actinomycosis.

LUYKEN (1939) discussed *Vaccinotherapy* of actinomycosis. According to reports collected from several veterinarians, 458 cases of actinomycosis in cattle and swine have been treated with a vaccine prepared with living apathogen acidfast bacilli of the *Timothee* group. According to the localization of the affliction from 50 to 90 per cent of the animals have been cured, in the average 80.7 per cent. The effect of the vaccine is probably an unspecific increase of the resistance and/or the effect of partial antigens. There is still a considerable diversion of opinion about the usefulness of this vaccine which may be due either to the difference of severity of the infection or to the variability of the organisms used for the preparation of the vaccine.

BANCROFT and STANLEY-BROWN (1938) surveyed the literature concerning the treatment of actinomycosis with *Thymol*. The foci of the disease have been located in 500 cases of actinomycosis; the head and the neck were involved in 55 per cent, the thoracic and the abdominal cavity were afflicted 20 per cent each. In the region of the appendix the actinomycosis appears as a painful tumor, as a gangrene, or as a perforation of the large intestines. As treatment, potassium iodide, copper sulfate, X-ray deep irradiation, and lately, thymol in crystal form by oral medication and as a 10 to 25 per cent oily solution for local therapy were applied. The latter has been also used for the drainage of wound cavities. The authors have had favorable results with thymol treatment in one case.

RANDALL (1939) discussed the pathology, diagnosis and treatment of *Actinomycosis* of the *Head* and *Neck*.

Of the various forms of treatment, surgery, radiation, and potassium iodide have proved to be the most valuable. HYERDAHL has reported good results with radiation therapy in actinomycosis of the head and neck, and prefers it in all types of actinomycosis to practical exclusion of all other therapy. MAGNUSSON has shown that actinomycosis in cattle is usually due to the Actinobacillus and that massive doses of potassium iodide have been effectual, but have had very little effect on lesions caused by the *Actinomyces bovis*. REYNOLDS and HENRICI have shown that iodides have no effect on actinomyces in vivo or in vitro. They obtained growth in media containing as much as 10 per cent potassium iodide. It was noted in several of the cases presented that, although the patients felt better physically, and the lesions did not seem to progress very much for a time, this manifestation was only temporary; the patients died from the disease. Therefore, it is doubtful that iodides exert any specific action on this parasite; and it is a question whether they stimulate fibrous tissue or not. It has not been possible to find any reported cases of complete cure of proved actinomycosis by the use of potassium iodide alone, or of radiation therapy alone, although some apparent remissions have been demonstrated.

In the treatment of actinomycosis, if possible, complete eradication of the diseased process by certain surgical procedures should constitute the chief therapeutic measure. For supportive measures, potassium iodide and irradiation therapy are of value, but they should be considered of secondary importance.

DE ALMEIDA and ABDALLA (1939) presented statistics on the occurrence and distribution of *Actinomycosis* in Brazil.

They collected 108 cases of this condition with the following distribution: upper extremities 68, lower extremities 17, cervicofacial 6, thoracic 4, abdominal 3, not specified 10.

RISCH (1939) presented a thesis on *Actinomycosis* of the *Ear*.

Actinomycosis involves the head and neck in nearly 60 per cent of the cases, according to WHALEN, but its incidence in or about the ear is relatively slight. A total of 31 cases have been reported in the literature. Analysis of the reports shows that in 16 cases (51.6 per cent) the condition was confined to the external auditory canal and the pinna; in 15 cases (48.4 per cent) it involved the middle ear.

Actinomycosis in man is not a common disease. It is approximately twice as frequently found in men as in women, and the incidence is greatest in the third decade of life. The distribution of the primary lesion throughout the body, according to LEITH's statistics, quoted by PUSEY, is: head and neck, 52 per cent; abdomen, 21 per cent; lungs, 13 per cent; tongue, 4 per cent; skin, usually a secondary involvement, 2.5 per cent.

In spite of the rarity of actinomycotic infection in otologic practise several factors justify an awareness of its potentialities as a primary invader and as a secondary complication on the part of workers in this field. In the past far too many infections have been discovered at autopsy or too late for effective therapy.

The WOLFF and ISRAEL strain of *Actinomyces* appears to be a strict parasite; it has never been found outside the animal body, and its survival in artificial culture is brief. These facts suggest that the bovine type is transmitted by infected pus, saliva or nasal discharge (TOPLEY and WILSON). The acid-fast species of *Actinomyces*, with other members of the genus, occurs as a saprophyte in soil or in vegetable matter and gains entrance to the body by being inhaled or through wounds (HENRICI and GARDNER).

A case of acute mastoiditis complicated by sinus thrombosis, septicemia and arthritis in which an aerobic acid-fast type of *Actinomyces* was isolated from the blood stream is presented.

It is suggested that, in spite of the rarity of actinomycotic infection in otologic practice, several factors justify an awareness of its potentialities as a primary invader and as a secondary complication on the part of workers in this field.

The method of treatment is still questionable in cases of infection due to the acid-fast types.

Both aerobic and anaerobic types of *Actinomyces* are capable of producing lesions in man, but it is impossible to distinguish the pathogenicity of the types bacteriologically.

It is suggested that the organisms gain entrance to the middle ear (1) through the eustachian tube or (2) by continuity.

SCHUERMANN (1939) investigated the clinical usefulness of bacteriologic findings of *Actinomycetes* with a contribution to the flora of the integument.

He came to the following conclusions. Anaerobe actinomycetes are constantly present on the integument, particularly in certain parts of the follicle. The positive finding of actinomycetes is no evidence in itself for their being the causative agent in the given case. This possibility has to be considered to avoid a false diagnosis of actinomycosis. The question is discussed whether saprophytic anaerob actinomycetes may turn pathogenic and thus, they are able to complicate already existing syndromes. They believe that there are certain atypical skin syndromes possible elicited or at least complicated by the presence of these micro-organisms. The "micro-bacillus of seborrhea," the "acne bacillus" (UNNA-SABOURAUD) is an anaerob actinomycetes.

WIEDERKEHR (1939) discussed the *Epidemiology* of *Actinomycosis*.

This investigation demonstrates that the problem of actinomycosis is by no means completely solved yet. Only its etiology and pathogenesis seem to be cleared up, its epidemiology, however, is still obscure. A survey of the literature and the analysis of the histories of 152 cases examined at the Hygiene-Institute of Zurich reveal the difficulties of a bacteriologic diagnosis of actinomycosis. All attempts to adopt anaerobic strains (WOLFF-ISRAEL) to growth in aerobic media have failed. Epidemiological inquiries in respect to age distribution, occupation, housing etc. did not furnish any link with free living aerobic varieties as the cause of human infections. The high incidence of dental caries as compared with the low case incidence in actinomycosis speaks against the assumption that pathogenic actinomyces are frequent inhabitants of the human buccal mucosa. No explanation can be offered for the difference of frequency in male and female (2/3 male, 1/3 female).

Emmons (1939) reported on isolation of *Actinomyces bovis* from *tonsillar* granules.

In an unselected series of 200 pairs of tonsils from routine tonsillectomies a micro-aerophilic species of *Actinomyces*, not distinguishable from *A. bovis*, was observed in 37 per cent of the pairs; it was obtained in culture from 11 per cent. None of the patients had actinomycosis.

These tonsillar strains, like those from clinical actinomycosis, are avirulent for guinea pigs. Repeated inoculations of the same animal, however, increased the severity and duration of lesions produced.

Sensitization to homologous and heterologous strains and to old tuberculin could not be demonstrated in experimental guinea pigs.

It seems probable that *Actinomyces bovis* is commonly present in the normal mouth and throat, and becomes pathogenic only under extraordinary conditions. The tonsillar crypts serve as an important reservoir for the saprophytic phase of this pathogenic fungus.

Dosa (1939) investigated the pathogenicity of *Nocardia bovis.*

Nocardia fungi were successfully cultured in a fair number of forms. Those cultivated from Actinomycosis pedis and primary actinomycosis of the skin were pathogenic for guinea pigs only.

The other forms found in different skin eruptions proved to be harmless for test-animals though there was no difference in morphology from the pathogenic forms. The morphology of the author's forms did not change even in 3 to 4 years. It was not possible to change the aerobic growing into anaerobic. After the experience the Nocardia as aerobic form of the Actinomyces can be pathogenic for the animal as well as the human organism and they form the same regular "granules" as the anaerobic species. The forming of granules is characteristic. The granules are not only forming in the organism but also in the hanging-drop. In man the various species of *Nocardia* have a different pathogenic effect. There may be fatal or there may be only slight changes. Frequently only saprophytes occur. The pathogenic action has no relation to the morphology of the species.

Franklin (1940) described a new species of *Actinomyces*, pathogenic for man.

In 1925, Sanford and Voelker reviewed 670 cases that had been observed up to that year in the United States, and of these only 2 per cent showed generalized involvement. Brumpt states that in those infections caused by *A. israeli*, the etiological agent of lumpy jaw in cattle, 60 per cent of the cases are of the cervicofacial type. When all causative species are grouped together this percentage is much greater. In certain cases decaying teeth may contain the organism and as such may be the source of the infection. Topley and Wilson state that *A. graminis* Bostroem is occasionally present in actinomycotic lesions and is probably not etiologically related to the disease. This organism, like the one herein later described, has been found in the mouth.

The organism described is a new species. It is proposed to call it *Actinomyces Moormani* after the technician herein previously mentioned because she was the first to differentiate it from a corynebacterium.

It is pathogenic to man, forming chronic abscesses.

Like *A. graminis* it is probably found in the mouth as a saprophyte.

For the purpose of identification of an actinomyces it is suggested that the media and procedures mentioned herein are all necessary and useful.

Media	A. graminis	Actinomyces sp. nov.
Gelatine	Liquefaction unusual.	Rapid liquefaction.
Broth	Pinkish, yellowish or orange in color. No odor.	None but chalky white. Odor distinctive.
Glucose agar slant	At three weeks, brick red or yellowish orange.	No such color. Blackish color below surface diffusing a few millimeters into medium.
Loeffler's serum	No liquefaction 24 days.	Rapid liquefaction.
Egg slant	No liquefaction 24 days.	Rapid liquefaction.
Blood agar slant	No hemolysin (Topley and Wilson). Hemolytic zone (Waksman).	Rapid and early hemolysis.
Czapek agar	Becoming yellow and brown as it dries. Penetrates the medium.	Fine white, black spekled colonies along streak. Does not penetrate into medium. Later brownish.
Starch	Enzymatic zone 12—16 mm	No enzymatic action.
Odor	None mentioned.	Heavy penetrating, moldy, escaping through tube plugs.

L. S. Auster (1940) reviewed the subject of *Actinomycosis* with detailed clinical and autopsy reports. Human actinomycosis is a relatively infrequent but widespread disease which is prevalent more particularly in grain-producing areas of the United States.

The causative organism is an anaerobe of specific morphological, cultural and serological character.

The pathologic picture is characterized by longstanding productive inflammation associated with liquefaction necrosis and draining sinuses, although diagnosis is frequently obscured by intercurrent infection.

The clinical course is that of a wasting infectious process, similar to tuberculosis, with bizarre symptomatology depending upon the organs involved or the anatomical location of the disease. No tissue is immune.

The abdominal form is usually ileocecal in origin, and in its early state presents a clinical picture similar to acute appendicitis, ileocecal tuberculosis, or non-epcific intestinal granulomatosis. It may be associated with bacillary dysentery.

The genito-pelvic type is more common in females and may occur by direct infection of the pelvic generative organs without prior intestinal involvement.

Dissemination of the disease, while showing metastatic (probably hematogenous) involvement of the liver, occurs by direct extension to neighboring organs with production of mural intestinal lesions rather than by lymphatic drainage pathways. Lymph node involvement is usually the result of secondary infection by bacterial invaders from the intestine or skin wounds. Peritonitis is a frequent complication.

Four illustrative cases are presented showing varying forms of the disease with particular reference to the abdominal and genito-pelvic forms, of which one case each is reported in detail with post-mortem findings. No apparent source of infection was determined.

Mortality, in types other than the cervicofacial form, has been unusually high on account of lack of effective therapeutic measures.

Best results in the treatment of actinomycosis, at this time, probably may be obtained by combined therapy including the use of surgery, high voltage x-ray treatment, and both local and systemic administration of thymol.

H. H. CLEMENS (1940) reported of a case of *Actinomycosis* in a child with recovery following thymol therapy.

Human actinomycosis appears to be widespread over the world, as reports of cases of actinomycosis in the adult, as well as in the child, have been published in almost every civilized country. In the European literature at least one thousand articles have appeared (COPE). On the other hand, reports of cases of actinomycosis in the American literature are relatively uncommon. MURPHY, in 1885, seven years after ISRAEL's initial report in Germany, described the first human cases in America. Four years later RUHRAH collected a series of sixty-two cases in the United States. SANFORD and VOELKER's survey in 1925 embraced 670 cases of the disease that had occurred in this country (including Mayo Clinic's and Ruhrãh's cases). In 1931 FIGI and CUTTS gathered together a total of 450 cases of actinomycosis that had been studied at the Mayo Clinic. Rarity of the disease in man is indicated from an examination of the 141,064 admissions to the Louisville City Hospital from 1923 to 1938, where only six patients have been diagnosed as having an actinomycotic infection.

It is unusual to find the disease in a child. Accounts of the disease are found only in the larger referrence books (BRENNEMANN, Abt.). EDWARDS reviewed the literature and reported on five cases of actinomycosis in children which he had investigated. FIGI and CUTTS included fourteen children (3 per cent) in their large series. About 6 or 7 cent per of the patients in SANFORD and VOELKER's extensive review were children. The incidence of the infection in children below 10 years of age is generally given at about 3 per cent (COPE, BRENNEMANN).

Actinomycosis may affect the infant as well as the child. The youngest patient reported was a 28-day-old marasmic infant who died with an actinomycotic pulmonary abscess. A case of cervical actinomycosis in a 10-year-old colored male child herein reported is of interest because: The diagnosis was made before sinus formation occurred. The organism involved was isolated, cultured, and identified as the *Actinomyces bovis* (of the Wolff-Israel type). Sulfanilamide, as well as deep roentgen therapy, was ineffective in the treatment. Recovery apparently occurred under adequate thymol therapy.

GOTSHALK and WILEN (1940) reported on the occurrence of *Actinomycosis* in *Hawaii*.

Eleven cases of *Actinomycosis bovis* are reported. Of this group five were pulmonary, one cervicofacial, two were abdominal, one presented a perirectal abscess; one, an ulcer on the leg, and one, madura foot. Of this group all five with pulmonary involvement died, one patient with abdominal infection died, and the remainder recovered.

Actinomycosis in humans is apparently more common in the Hawaiian Islands than previously thought, and presents a definite medical problem.

De Almeida and Da Silva Lacaz (1940) reported on cervicofacial *Actinomycosis.*

Cervicofacial actinomycosis is rare in Brazil. They studied six cases in which diagnosis was made mycologically by direct examination of material obtained from the lesions. They successfully used the vaccinotherapy in this condition.

Dobson et al. (1941) reported on *Sulfanilamide* in the treatment of *Actinomycosis.*

Six clinical reports have mentioned the use of sulfanilamide or related compounds in actinomycosis, and one report describes a trial of the drug in an experimental infection. The earliest of these was that of Walker, who in 1938 reported a case of abdominal actinomycosis arising from the appendix, which healed quickly after two courses of sulfanilamide. The first course lasted six days and the second five days, with an interval of ten days. The dosage was 1 Gm. three times a day throughout. Compound solution of iodine and potassium iodide had been given previously without effect.

Bailey, also in 1938, reported a case in which potassium iodide, sodium iodide intravenously, thymol and sulfanilamide were administered. The report, made while the patient was still in the hospital, stated that he was improved but not well. The dosage of sulfanilamide was not mentioned.

Miller and Fell reported a case in which a mass in the lower part of the abdomen of a boy proved on incision to be actinomycotic. Thymol and potassim iodide were given orally, thymol in oil applied locally, and roentgen irradiation given as well, over a period of four months. The patient lost strength and the abdominal mass grew larger, however, and sulfanilamide was started in small doses, 0.65 Gm. three times a day. This was continued for ten months with apparently complete recovery.

Ogilvie recently reported a case of actinomycosis probably arising in the appendix and involving a large part of the anterior abdominal wall. Despite surgical opening of the sinuses and long continued administration of iodide, the infection advanced. Sulfapyridine, 2 tablets six hourly (this probably being 4 Gm. a day), was given with dramatic and permanent recovery. The drug was given for only thirteen days, average blood levels being 2 mg. per hundred cubic centimeters for the free drug and 3.3 mg. per hundred cubic centimeters for the total drug. The author suggests that the action of the drug was largely against the coincidental streptococcic infection and that the cure of the actinomycosis then resulted from natural processes.

Sudler and Johnson recently reported two cases of actinomycosis of the jaw treated successfully with sulfanilamide. One of the patients was getting worse while on iodides and roentgen treatments and then healed promptly after two short courses of sulfanilamide. The second patient was treated with sulfanilamide alone.

Morton reported 3 cases of actinomycosis, in 2 of which sulfanilamide was administered. In 1 there was involvement of the jaw, face, meninges and brain, and death occurred although sulfanilamide was given at one time for a period of a month in doses of 4 Gm. daily. In the other case there was actinomycosis of the breast and axilla, and sulfanilamide was given intermittently for about six weeks in doses of 4 to 8 Gm. daily, with blood levels of 4 to 8 mg. per hundred cubic centimeters. At the time of the report the patient was improved but not well. Both of these patients had iodides, roentgen rays and vaccine injections, as well as sulfanilamide, and the patient who died also had thymol applied locally.

The experimental report was that of HEMMENS and DACK, who found that they could cure otherwise fatal infections of rabbits with *Bacterium necrophorum*, an organism distantly related to *Actinomyces bovis*, by early and persistent treatment with sulfanilamide.

These favorable reports are in considerable contrast to the conclusion of GOOD, who in 1931 reported 62 cases of abdominal actinomycosis. Treatment consisted of free drainage, potassium iodide, general upbuilding and radium or roentgen irradiation. Only 8 were apparently cured, and it was concluded that the results were generally poor. More recently another report from the Mayo Clinic by MASSON included 2 cases treated with roentgen rays and small doses of iodide, with complete recovery. Mention is made of another fatal case, however.

Three cases of actinomycosis, one of the jaw, one of the lungs and rib cage, and one of the abdomen, have been apparently completely cured. Therapy included the use of sulfanilamide, iodides and roentgen rays. In view of the serious nature of this infection and the apparent value of sulfanilamide, this or a related drug should be included in the treatment of actinomycosis.

P. Experimental Biology and Immunology

WILLIAMS (1938, 1) investigated the *"Integrative action" of mixtures of simple organic substances in oxidation-reduction phenomena produced.*

The "integrative action", subsequent to autoclaving, expressed in persistence of reduction of methylene blue. Such action is divided into three types:

a. That of mixtures of organic acids and sugars which is more pronounced at pH 8.0 than pH 5.6.

b. That of mixtures of d-glutamic acid and cysteine HCl which is observable in solutions of pH 5.0 but not 8.0.

c. That of mixtures of d-glumatic acid, cysteine hydrochloride and glucose which shows a vacillatory state of partial reduction and partial oxidation in solutions of pH 5.0 but not 8.0.

WILLIAMS (1938, 2) reported on *Reduction and concentration of methylene blue by certain pathogenic fungi.*

Ability to concentrate and reduce methylene blue is of importance in differentiation of certain pathogenic fungi. To study concentration, a medium producing a predominantly subsurface growth (0.25 per cent d-glutamic acid, Hoffman-LaRoche, 4 per cent glucose Difco, 1.5 per cent agar Difco, pH 5.6) is preferable. To study reduction, either the above medium or a medium producing a predominantly surface growth (Sabouraud's medium Difco) may be used. A 0.001 per cent concentration of the dye (methylene blue, U.S.P. medicinal, CI 922, National Aniline & Chemical Co.) is ideal.

Surface plants on 4 to 5 cm. straights of the above mediums were made and observed after 5, 7, 10, 15 and 30 days growth in diffused light at room temperature with tubes secured upright. Three or more plants of each organism were made, since, otherwise, failure of an organism to cover the surface or cracks in the medium rendered results less accurate. All reduced areas were checked by stabs whereupon color reappeared. Natural organismal pigment was discounted in tabulation of results.

Dye concentration occurs only in the upper 2 cm. of the straight in proximity to, and in, certain growths (area of increased metabolism). This correlates with localization of acid dyes injected into animals in areas of inflammation (BURROWS, 1932). The dye line occurs at the base of the dye concentration or independently. Pronounced concentration and dye line occur in a similar situation in cysteine medium after autoclaving but when other amino-acids are substituted, only after growth of certain organisms. Successive dye lines similar to Liesegang rings occasionally occur. Subsurface growths do not occur at a depth greater than 2 cm.

The green frequently noted may be a combination of changed dye and yellow resultant from alkaline change in the mediums. The lavender, violet or purple occur at a definite time (10th day) and therefore seem biologically fundamental in time. Basically the various color changes seem dependent on combinations of oxidations and reductions of the various constituents.

Reduction time and its completeness varied with the organism. Twenty plants on W medium (4 per cent peptone, 1 per cent glucose, 1.5 per cent agar, pH 5.6) indicated that this ratio of peptone to glucose speeded reduction, possibly because of better buffering power of the medium (WILLIAMS, 1935 and 1936a).

WILLIAMS (1938, 3) investigated the effect of *Surface tension and osmotic pressure on the gross morphology of certain pathogenic fungi.*

Surface tensions were taken on broths of mediums used with the du Nouy interfactial tensiometer at the airbroth interface; two minutes were allowed for stabilization after pouring.

Osmotic pressure was raised by adding salts to W medium. The moles per liter of NaCl or KI added (resultant osmotic pressure of medium in atmospheres at 20° C. in parentheses) were: NaCl (a) 0.1 (11,) (b) 0.3 (21), (c) 0.5 (31), (d) 1.0 (55), (e) 1.5 (79), (f) 2.0 (104), (g) 4.0 (199); KI (h) 0.1 (11), (i) 0.3 (21), (j) 0.5 (31).

Increasing concentration of peptone, adding sodium taurocholate [1], Brilliant Avirol L-124 or Gardinol Ca to W or S medium decreased surface tension and produced or augmented the following appearances: colony delimitation, radii, frosting, wrinkling, raised appearance, waxiness, wetness, spicule, spike-like or whiskery mycelia. By lowering the surface tension of the S medium to that of the W medium duplication of W medium morphology was only partial. Findings indicate that surface tension may be an important factor in results.

Increasing osmotic pressure by adding sodium chloride or potassium iodide bore a proportionality relation and produced or augmented the following appearances: colony delimination, raised appearance, irregularity of colony, waxiness, wetness and to a lesser degree other changes cited for decreased surface tension.

The least evaporation of moisture was from W medium containing 2 moles sodium chloride per litre; the amount was one-fourth less than for W medium. In view of other studies (WILLIAMS 1937c) this does not seem sufficiently great to explain results.

Certain organisms (*Candida candida, Geotrichum bachmann,* etc.) were stable to the effects of any of the agencies used.

Similarity of the progression of changes with decreasing surface tension and increasing osmotic pressure suggest a common underlying factor. This progression is evident: subsurface growth, surface growth, raised growth, first smooth then radiate, then irregular and lumpy growth. Previous studies have shown that on certain mediums (WILLIAMS, in publication) and with certain organisms sodium lactate and sodium phosphate can accomplish similar changes. Likewise, on certain mediums decrease in oxygen tension can produce some of these variations. In both of the latter instances surface tension and osmotic pressure do not seem to play a part.

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[1]) Abbreviations are used: P, peptone Difco; D, glucose Difco; NT, sodium taurocholate Merck; GC, Gardinol Ca Dupont; BA, Brilliant Avirol L-124 Dupont; S, Sabouraud's medium of 1 per cent peptone, 4 per cent glucose; W, W medium of 4 per cent peptone, 1 per cent glucose; c, with.

In the use of the various agencies mentioned some organisms come to resemble each other. This may indicate a fundamental biological relationship.

Similarity of changes produced by increasing osmotic pressure and decreasing surface tension is responsible for inclusion of both problems in the same paper. Both seemingly accomplish a similar sequence of events. Whether the sequence has a physico-chemical basis can only be explained by further study.

WILLIAMS (1938, 4) reported on *Bacterial growth "spectrum" analysis.*

When certain bacteria are grown in shake cultures of specified mediums under greatly increased oxygen pressure they develop characteristic bands of growth varying in width, number, density and type, and often separated by clear zones. These appearances simulate in some degree the bands in a spectrum and the author, therefore, has given them the name "bacterial spectrums". "Bacterial spectrums" vary in width and depth dependent on oxygen tension, the composition of the medium and its concentration. The majority of organisms studied have shown marked constancy in appearance, although changes in pH from 7.2 to 5.6 and variations in quantity of inoculum of 24 or 48 hr. culture from 0.1 to 0.3 cc. have produced minor variations in a few species.

The medium is put up in 12—15 cc. shakes in bacteriological test tubes. Wasserman tubes may be used with proportionately less medium. The melted agar medium (0.75 % agar throughout) is planted at 40—50° C. with 0.1 to 0.3 cc. of 24 or 48 hr. broth culture in duplicate. Tubes are immediately rolled several times between the hands and plunged into running tap water to solidify. Incubation is for 48 hrs. under a pressure raised to 60 lbs. with oxygen.

The oxygen apparatus consists of a heavy iron pressure chamber, of a size to fit the incubator and provided with suitable attached gauge and valve for regulating gas. Oxygen is provided by a commercial tank. Care is taken that no oil is present in the apparatus since oil and oxygen may result in an explosion. Adjusted lids and valves must hold pressure for 48 hrs. if results are to be reliable. The apparatus is so simple that frequently the parts can be picked up in the laboratory and fitted by a machinist.

On removal from the chamber the depth of the "spectrum," its width, position and width of bands are measured, the growth is described and the "spectrum" photographed against a lighted, frosted glass background. A roll film camera with portrait lens so adjusted that a 1 : 1 image can be recorded is used. Black paper is pasted over the back of the camera (film holder removed) so that only a narrow slit of light contacts the film at each exposure. In this way by turns of about an inch of film 40—60 "spectrums" can be recorded to the film.

The following mediums all containing 0.75 % agar and adjusted to pH 7.2 have been studied: (a) 0.2 % nutrient broth Difco, (b) 0.1 % nutrient broth Difco, (c) 0.05 % nutrient broth Difco. Medium (a) with each of the following 0.0005 % methylene blue, 1 % sodium chloride, ½ % sodium lactate, ½ % monobasic sodium phosphate, 0.1 % sulfanilamide Merck, 1 % cysteine hydrochloride Eastman also has been employed.

The following organisms have been studied: *E. coli communis*, *B. subtilis*, *Staphylococcus aureus, Sarcina lutea, Serratia marcescens, Ps. fluorescens, Ps. aeruginosa, Aerobacter aerogenes, B. megatherium, Proteus vulgaris, B. niger, B. mycoides, Staphylococcus albus, Eb. typhosus, Proteus X*-19, *B. anthracis* (nonvirulent), *B. Flexner, B. Shiga, S. paratyphus, S. schottmulleri, S. suipestifer, S. enteritidis.*

The aerobic nature of organisms with reference to a medium can be judged by allowing shake cultures to incubate at atmospheric oxygen tension. Some organisms grow only on the surface, others show a smooth surface with growth just below the surface. The latter often grow with difficulty on ordinary streaks owing to too much oxygen since unlike fungi they are unable to invade the medium to find their optimum oxygen tension. Such difficulty of growth sometimes may result in abnormally scanty development and pleomorphic forms, a fault which can be corrected if optimum medium-oxygen relationship can be established at this site. Again, oxygen tension may not be adequate, in which case changes should be in an opposite direction.

Depth of spectral growth increases with increase in oxygen tension, and with decrease in concentration of medium for all organisms studied. Addition of certain substances as methylene blue (hydrogen acceptor) gave similar results for certain organisms studied. Width of spectrum (density of growth decreases) increases with decrease in concentration of medium; frequently this increase in width reveals new bands. Depth of spectrum decreases for reverse reasons and for certain organisms in certain mediums by addition of substances as cysteine (hydrogen donator). Three factors, determine variation (a) change in oxygen tension, (b) change in growth range (it remains to be proven if this is due to widening of optimum oxygen tension or/and Eh), (c) specific substance effect (it remains to be proven whether this is a hydrogen donator and hydrogen acceptor effect in a specific organism system). Therapeutic efficiency of drugs possibly may be explained in certain instances by specific substance effect demonstrable in the test tube. Our tissues are mediums and each has its oxygen tension. Organisms demand a certain oxygen-medium relationship for growth; if drugs change this in infected tissues further growth and harm thereform should be obviated.

WILLIAMS (1938, 5) investigated the *Difference in growth of pathogenic fungi with variation of medium and oxygen tension.* The atmosphere-medium relationship determines amount, type of growth, and surface or subsurface tendencies of microorganisms.

Invasion of medium results from the organism seeking the most favorable atmosphere-medium relationship for growth and departure of growth. Subsurface tendency apparently bears a proportionality to the anaerobic nature of the organism.

Inhibition of growth claimed by other workers for high oxygen tensions should be decided only after growth weight studies.

Partial oxygen tension determines the depth and presence of growth on a specific medium.

Sparse superficial or no growth on SABOURAUD's medium under 1 per cent oxygen often results in death of the organism.

Invasion of tissues and selectivity for tissues in disease are undoubtedly dependent on the medium (tissue)-atmosphere (oxygen tension) relationship and pathogenicity on the added injury through the organism. Simple factors may change this relationship entirely.

WILLIAMS (1939, 1) investigated the *Factors of importance in susceptibility to disease.*

Susceptibility to disease is considered from the standpoint of the body furnishing mediums for the growth of micro-organisms. The importance of the atmosphere and method of dissemination of the organisms is considered.

The cause of inflammation is discussed. The writer questions one isolated substance as basic. He considers numerous factors may disturb the cell layer increasing permeability and that reaction of substances from the inside of the cell with those on the outside may have a similar effect on neighboring cells and so on until the reaction is exhausted.

The importance of equilibration in the life of the body and its cells and the existence of all things is considered in detail. The importance of rhythmicity in the existence of man and all things is emphasized.

Incidence and variation of disease with age, sex and structure is considered. Basic factors which may be explanatory of variations are correlated.

Correlations with test tube experiments growing micro-organisms on various mediums are made. Concentration of dyes, effects on mediums, spreadings and contraction of organismal growths, reductions and oxidations, osmotic pressure and surface tension are discussed in relation to their possible importance in explaining the sequence of events in inflammation.

Allergy is discussed as inflammation. The incubation period of disease is visualized as an accumulation of allergic antibody in cells to the point of saturation at which point it is discharged into the circulation, contacts areas where the organisms are proliferating or in focus with a reaction which is characteristic of the onset of disease.

The possibility that skin of the immune may contain constituents, for example, of metals and salts in sufficient concentration to be inhibitory to growth is suggested on the basis of experimentation now being carried on.

Five factors are important in production of infectious disease in the animal body: (a) The micro-organism; (b) the medium furnished; (c) the atmosphere; (d) the means of dissemination; and (e) the reactions of the body (or the medium). They are interdependent, one on the other.

Certain physical measurements can be taken of the medium and its organism and of the area of disease. The exact significance of some of these measurements with reference to inflammation and in organismal growth is unknown. The reliability of some of these measurements is often question. A few suggestions as to possible significance of some of these variations are made in this paper.

WILLIAMS (1939, 2) reported on *Method for determining suitability of solid medium for growing anaerobes under aerobic conditions with an example.* A method of obtaining specimens at various levels in shake mediums for Eh and pH measurements is described.

The observation is made that there is a variation in Eh at various depths of unplanted medium: with increase in alkalinity Eh becomes more negative up to a certain point with depth: with increase in acidity this change becomes less. A frequently observed similar Eh at several levels suggests a possible explanation of bands of growth.

Theoretically, knowing the Eh at various depths in a medium, that area at which an organism will grow should be predeterminable: knowing the Eh over which an organism grows, the area of growth should measure the Eh in the respective medium.

Varying Eh in mediums containing only agar gives added proof of its dynamic nature in growth of microorganisms.

Increase in oxygen tension seems to lower the optimum Eh area of a medium for growth and likewise the growth.

Growth of anaerobes on the alkaline medium used is discussed. In many instances the alkalinity attained is well above the optimum given in various texts. The growth, however, is good. The good points of this medium are discussed in some detail.

The relation of aerobes to this problem is discussed.

WILLIAMS (1939, 3) investigated the *Growth of microorganisms in shake cultures under increased oxygen and carbon dioxide tensions.* Depth in upper limit of spectral growths varies with time of growth, oxygen tension, carbon dioxide tension, temperature of incubation, number of organisms planted, amount of nutrient, growth inhibiting factors, reducing and oxidizing agents.

Width of spectral growth varies with time of growth, percentage of nutrient, growth inhibiting factors, reducing and oxidizing agents, organisms establishing growth, temperature of growth, substances encouraging anaerobic growth.

Consistency of spectrum varies with time of growth, percentage of nutrient, growth inhibiting factors, reducing and oxidizing agents, organisms establishing growth, temperature of growth, substances encouraging anaerobic growth.

Within limits oxygen counteracts inhibiting effects of carbon dioxide.

Eh and pH of medium above spectrum are similar to controls but decrease usually occurs in and below the growth to a varying extent and depth with certain organisms. Changes in bromthymol blue and methylene blue included in mediums are what would be expected from Eh and pH readings.

All organisms studied are capable of concentrating methylene blue to a variable extent in lines and bands in and directly above their spectrums and some are able to change the blue to its various azures.

Explanation of the various changes is suggested. Suggestive evidence is presented indicating a gradient of oxygen diffusion in mediums studied.

Most appearances produced with inanimate precipitates in gels have been produced by bacterial growths in shake cultures.

It is felt that the great possibilities presented by growing microorganisms under increased gas tensions should win this method a place with anaerobic and aerobic methods of growth in bacteriological investigations.

WILLIAMS (1939, 4) investigated the *nature of gel mediums as determined by various gas tensions and its importance in growth of micro-organisms and cellular metabolism.*

Data is given in charts which illustrate trends and practicability of the author's method of study of Eh and pH in gels for learning more about gels so that their function and the function of ingredients they contain may be better understood in investigations of living tissues and organisms which grow in and upon them.

Data is given illustrating the effects of gases and tensions of gases on gels and on microorganisms growing on and in them.

Correlations of the above studies with the skin and mechanism of respiration are suggested. Similar correlations can be made with other living systems.

Suggestions as to possibilities in further investigations are presented.

Suggestions as to explanations of variations noted in growths on mediums sterilized and prepared differently are given. The ability of mediums to retain oxygen in contradistinction to their inability to retain hydrogen is discussed.

It must be realized that there are many variables in experiments such as these. The author has attempted to give generalities in which the variables seem to have been satisfied as a point of departure for further studies.

A discussion which indicates that all changes noted may be dependent on "adsorbed" and "unadsorbed" oxygen is presented. Possibly too many, although pertinent, assumptions have been made in this discussion.

GRIGORAKI and DAVID (1939) investigated the *biochemical characteristics of Endodermophyton indicum*, Castellani 1911. The investigations on different mediums (milk, gelatine, different carbohydrate solution with lithmus) have shown the following results: this fungus showed a less effective *casease*, but a very strong *trypsin* already at a minimal growth. Carbohydrates (glucose, mannose, galactose, saccharose, lactose, maltose, inulin, dextrin) and glycerol have been attacked only slightly. The biochemical characteristics of this fungus reminded that of *Achorion violaceum* with which this fungus is closely related.

HOLLAENDER and EMMONS (1939, 1) investigated the *fungicidal* and *sublethal effects of monochromatic ultraviolet radiation on the spores of dermatophytes.*

Spores of *Trichophyton mentagrophytes* (a strain causing "athlete's foot") were irradiated with measured quantities of monochromatic ultraviolet radiation between the wavelengths 2180—3550 A in such a manner that each spore received and absorbed with practical uniformity an equal amount of monochromatic radiation during a given exposure. Percentage survival ration curves were plotted from the data obtained by plate counts. Wavelength dependence curves for 50 per cent survival ratio of colony-forming organisms at the wave-lengths between 2,280—3,000, the region most thoroughly tested in this investigation, show a definite maximum at 2,650 A, and a decreasing sensitivity towards lower and higher wave-lengths. The sensitivity of these spores is about 100 times less the sensitivity of typical bacteria and is close to the sensitivity of yeast cells. The first sublethal effect observed was a delay in the appearance of colonies produced by spores surviving irradiation. This is emphasized by the appearance of some colonies of smaller size and very slow growth; this physiological effect disappears in subsequent transfers. — A certain percentage of spores which had survived large amounts of radiation produced colonies which differed from the normal in colony form, color, rate of growth, and spore production. The appearance of these variants was regular, persisted in consecutive transfers, and was most pronounced at certain energy values and wavelengths. The wavelengths most effective in killing, 2,650 — 2,537, were also most effective in producing these variants. The absorption spectra of possible compounds in the cell nucleus affected by the radiation will be discussed.

HOLLAENDER and EMMONS (1939, 2) investigated the action of *ultraviolet radiation on dermatophytes* with regard to the *fungicidal effect of monochromatic ultraviolet radiation on the spores of Irichophyton mentagrophytes.* Spores of a dermatophyte (*Trichophyton mentagrophytes*) isolated from "athletes' foot" were suspended in a physiological salt solution, non-absorbent for the wave lengths used (2280 to 2950A) and exposed to measured quantities of monochromatic ultraviolet radiation. The method of preparing the suspension is described.

Inactivation data for six wave lengths in the ultraviolet range indicate that 2537 to 2650A is the most effective region for the fungous spores tested.

Data for 50 % inactivation of the strain used in most of the tests, and for four other dermatophytes show considerable uniformity for the first strain, and a wider variation for the other strains, which for reasons mentioned were not suitable for experimental material.

The possible significance of the inactivation maximum at 2537 to 2650A in its relation to the absorption spectrum of nucleic acid is discussed.

EMMONS and HOLLAENDER (1939, 1) investigated *the action of ultraviolet radiation on dermatophytes.* In this investigation they have dealt with *mutations induced in cultures of dermatophytes by exposure of spores to monochromatic* *ultraviolet radiation.* A method of exposing a suspension of spores of dermatophytes to monochromatic ultraviolet radiation is described. The energy was measured so that the amount expended on each spore can be calculated.

When spores of a strain of *Trichophyton mentagrophytes* are exposed in this way, besides the lethal effect, interesting secondary effects are observed, the most striking being mutant production.

Both mutant production and lethal effect are greatest after exposure in the region of wavelengths 2,537 and 2,650A.

Rate of mutations reaches a maximum at certain energy levels (in the case of these spores when exposed to radiation of wavelength 2,650A, the maximum is 100×10^4 ergs per spore) and rapidly decreases with increasing amounts of energy (increased time of exposure).

The mutans are placed in six arbitrarily delimited groups. The majority of them show an increase in pigment production and a decrease in growth rate. A few of them are indistinguishable from other species or other varieties of dermatophytes. The mutants are comparatively stable.

No mutants appeared in 2,000 control colonies isolated from nonirradiated spores. However, when spores from an old culture of the original strain were isolated, several mutants appeared, some of them being similar to those induced by ultraviolet radiation. Ultraviolet radiation merely accelerates the rate of mutation.

The significance of mutation in the taxonomy of dermatophytes is considered.

Emmons and Hollaender (1939, 2) investigated the *influence of monochromatic ultraviolet radiation on the rate of variant production in Trichophyton mentagrophytes.*

Trichophyton mentagrophytes, a dermatophyte classified among the *Fungi Imperfecti* (no sexual stage known), can be considered as typical of the less highly developed fungi. It produces uninucleate spores, which, in this investigation, were isolated from the multinucleate cells of the microorganisms, and exposed to measured quantities of monochromatic ultraviolet radiation. By random sampling and transfer of germinated spores to agar slants it was found that some of the spores surviving radiation had been so altered that heritable differences from the parent type appeared in cultures. The variants produced by the radiation can be roughly separated into four groups. All of these types differ from the variant commonly appearing in old cultures ("pleomorphic" growth) and are easily distinguishable from abnormal colonies due to adverse growth conditions. The uniformity of consecutive subcultures of normal strains of dermatophytes when properly cared for, and the apparent permanency of the variants induced by ultraviolet radiation indicate that under controlled environmental conditions colony form, growth rate, and pigment formation are inherited characters of genetical importance.

Castellani (1939) investigated the *viability of some pathogenic fungi in distilled water.*

On July 5, 1938, thirteen tubes of sterilized distilled water were inoculated with the following fungi (each with one fungus):

Monilia (Candida).

Monilia krusei Cast.
Monilia pinoyi Cast.
Monilia tropicalis Cast.
Monilia pseudotropicalis Cast.
Monilia macedoniensis var macedoniensoides Cast.

Geotrichum.

Geotrichum rotundatum Cast.
Geotrichum matalense Cast.
Geotrichum asteroides Cast.
Geotrichum rugosum Cast.

Miscellaneous.

Epidermophyton floccosum Harz.
Cladosporium mansoni Cast.
Aleurisma castellanii Pinoy (— Acladium castellanii).
Actinomyces sylvodoriferus Cast.

The distilled water test tubes were inoculated from glucose-agar cultures, taking care not to transfer to the liquid particles of the glucose-agar. The tubes were sealed at the flame and kept at the temperature of the room in the mycological laboratories of the London School of Hygiene and Tropical Medicine until July 10, 1939. On that day the tubes were opened, breaking the necks after filing, and, after shaking, inoculations were made in glucose-agar tubes.

Growth developed in all the glucose-agar tubes, within the normal time, and the macroscopic appearances of the cultures were typical, as can be seen from the photographs. The *Monilias* were passed through the usual carbohydrates (glucose, laevulose, maltose, galactose, saccharose, lactose, inulin), and gave the typical specific gas-fermentation reactions. The strain *Monilia pinoyi* produced gas-fermentation in glucose, laevulose, and maltose; *Monilia krusei* in glucose and laevulose; *Monilia tropicalis* in glucose, laevulose, maltose, galactose, saccharose; *Monilia macedoniensis* var. macedoniensoides in glucose, laevulose, galactose, saccharose, and inulin; *Monilia pseudotropicalis* in glucose, laevulose, galactose, saccharose, inulin and lactose. An investigation of the microscopical characters is being carried out at the present time.

The strain of *Epidermophyton floccosum* inoculated into distilled water on July 5, 1938, was the old laboratory strain which became partially pleomorphic several years ago, being fluffy but still showing a certain amount of the characteristic canary yellow colour. The culture made on glucose-agar after twelve months in distilled water showed the same partial pleomorphism with some characteristic yellow color present.

From the amount of sediment in the inoculated distilled water tubes one has the impression that several of the fungi must have slightly grown (*Geotrichum matalense, Geotrichum asteroides, Monilia tropicalis*). This is certainly the case with regard to *Cladosporium mansoni.*

NEGRONI and QUIROGA (1940) made interesting observations on *allergic manifestations* with the clinical picture of *Pityriasis marginata* and *circinata* of *Vidal.* They observed two cases of cutaneous involvement of an allergic nature. One showed the clinical picture of *pityriasis circinata* and *marginata* of VIDAL, and the other revealed erythemato-squamous lesions on the forearms. They assumed that these dermatoses are elicited by sensitization due to the presence of yeastlike organisms in the intestines. This etiology was proved by the presence of hypersensitivity to an extract made from *candida albicans.* Intradermo-reaction, complement fixation and, in one case, passive transfer (PRAUSNITZ—KUSTNER) were positive. They considered as the sole sensitizing focus the presence of yeastlike organisms of the genus *Candida* in fecal matters. The final proof of their etiologic assumption seemed to be the successful cure of the affection, the elimination of the parasitic focus without any local treatment of the involved areas of the skin.

HENRICI (1940) discussed the immunobiologic characteristics of *fungous diseases.*

The outstanding characteristic of the fungous diseases is their marked tendency to give rise to hypersensitivity of a type best designated as the allergy of infection, distinct from other categories of hypersensitivity. The other characteristics of the mycoses, age and occupational incidence, course and pathology, are strongly colored by or dependent upon this allergy. This characteristic distinguishes the fungous diseases from most of the other microbic diseases, but closely relates them to tuberculosis, and in some aspects to leprosy, syphilis, glanders, tularemia, and lymphogranuloma inguinale. These diseases present a common problem in immunology more or less peculiar to themselves. This characteristic is determined by the ability of the pathogenic fungi and related microbes to produce peculiar antigen-like substances having specific properties not common to all antigens, and that the deep-seated mycoses are caused by occasional species of saprophytic fungi which, evolving in restricted areas, have by chance acquired the ability to synthesize such substances.

WALDBOTT and ASCHER (1940) reported on Rust and Smut, *major causes of respiratory allergy.*

Smut fungi occur on a wide range of hosts, the most common ones being the cereals and grasses. In this part of the country (Detroit, Michigan) the most important species are the stinking smuts of wheat (*Tilletia triciti* and *levis*), the smut of corn (*Ustilago zeae*), and that of oat (*Ustilago levis*). Smut spores infest the plant of the grain by attacking its fruit where they develop black, dusty masses.

The growth of rusts is more complex than that of smuts since rust requires two hosts for the completion of its life cycle. Rust spores infest the stems, not the fruit, of the various grains, grasses, and fruit trees. They have been particularly concerned with grain rusts (*Puccinia graminis*), especially those of wheat and barley. They were also studying rusts of grasses, especially of timothy and blue grass.

The spring stages of the wheat rust occur upon the leaves of the common barberry bush, the summer and winter stages on the stems of the wheat plant.

Since July, 1938, they have charted the number of rust and smut spores found on the vaseline-coated slides which were exposed for 24-hour periods for the routine pollen counts. The firsturediniospores of rust occurred about July 15 and reached a rather high peak about July 21, on which day 149 spores were counted. The peak on July 30 is probably attributable chiefly to smut, but there was still a small number of rust spores on the slides during the early part of August. *Alternaria* was present throughout the time of observation and continued late into the fall.

A daily slide survey was made for spores of rust and smut. The curve exceeded in height the peak of the timothy curve and reached its maximum about July 23 and 30. The spore curve did not reach the height of the ragweed curve. Among 106 consecutive cases of asthma and upper respiratory allergy, seven had symptoms exclusively at the rust and smut season, and 12 patients showed more or less severe exacerbations at that time. The average intradermal skin reactions for rust and smut in these patients were compared graphically with reactions to other important antigens. Considerably stronger reactions to rust and smut occurred in the first two groups of patients, who exhibited symptoms at the time in question only. In the two cases here reported attempt was made to reproduce asthmatic attacks by inhalation of rust; this was accomplished without difficulties. On the basis of the above findings, they consider sensitivity to rust and smut an important cause of seasonal allergy of the upper respiratory tract.

Schonwald (1941) made investigation on *fungus allergies.*

Forms of fungus allergy follow the pattern of all inhalatory allergies except for the fact that one encounters a greater number of skin afflictions than are commonly seen in pollinosis or dust allergy cases. Among the 384 cases where mold allergy was complicated by sensitization to foods or pollen or dusts,

	Per Cent
18 cases of asthma	49
110 cases of rhinitis	29
16 cases of bronchitis	4
65 cases of skin allergy	17

Among 34 cases of uncomplicated mold allergies:

6 cases of asthma	17
8 cases of rhinitis	23
5 cases of bronchitis	15
15 cases of skin allergy	44

A discussion of the problem of fungus allergy is presented. The important fungi in the region of the western slope of the Pacific Northwest belong to the species *Alternaria*, *Aspergillus*, *Hormodendrum*, *Penicillium* and *Trichoderma.* A great number of skin allergies are the result of fungus disease, especially so in cases of trichophytosis and epidermophytosis. Skin testing and desensitization treatment are done with extracts of the fungus spores alone, with the exclusion of mycelia. The majority of fungus allergy cases are complicated by sensitization to other allergens. Only by proper elimination and/or desensitization to all pertinent allergens can successful treatment be achieved. Cases of uncomplicated fungus allergy obtain immediate and lasting relief if treated with the proper fungus extract.

Q. Aspergillosis

Fazakas (1938) reported on investigations about the role of common molds in the cause of diseases of the eye. In 456 *normal* eyes examined he found certain common molds in 25.6 per cent of the cases. It was found: *penicillium* 36 times, *torula* 18 times, *aspergillus* 5 times, *schizosaccharomyces* 14 times, *actinomyces* twice, and moreover 12 unclassified fungi. In 37.8 per cent of eye afflictions a great variety of molds were registered. Particularly, in cases of dermatitis of the face and eyelids common molds were found in pure cultures.

Hay (1939) published an interesting and, in many respects, clarifying investigation on *arsenical wallpaper poisonings* or *aspergillosis.* The literature of science in all of its branches includes many instances in which conclusions reached by eminent authorities of past years have been refuted on the basis of further investigations. Many times their opinions have been found to be the direct opposite of the real truth. The present paper expands on a paragraph written by Gosio in 1901 and proposes a re-examination of the cases diagnosed as arsenical poisoning from wallpapers.

For over one hundred years there appears to have been a confusion of thought on the part of doctors, chemists, and botanists with regard to certain cases of poisoning ascribed to arsenical wallpapers. As early as 1815, references began to appear which attributed a poisonous action to the arsenic contained in colored wallpaper, hangings, carpets, and certain paints. In many instances, the arsenical compounds were most loosely bonded to the material they colored and could be brushed off with comparative ease. This gave rise to the first explanation of the deaths and illnesses; namely, inhalation of pigmentparticles suspended in the air in dust form.

Beginning in 1839, a new theory for the poisonings was introduced. GMELIN, in that year, ascribed the cases to the poisonous action of volatile arsenicals liberated from disintegrating wallpaper in damp rooms and observed a garliclike odor in these rooms.

The toxic gas theory has been widely accepted because it was believed to explain certain illnesses which could not be imputed to a dusting-off of the pigment.

One of the first systematic studies, yielding a positive explanation of the garlic-like odors observed in the rooms, was that of GOSIO published in 1892. GOSIO proved conclusively that arsenic in most of its solid compounds could be attacked by certain moulds, if in the presence of carbohydrates, moisture, and oxygen. Under these conditions a biological reaction occurred which caused the liberation of a garlicky gas. He proved that the gas contained arsenic, considered it to be an organic derivative of arsine and further found that with the organism *Penicillium brevicaule* the reaction was extremely sensitive, and suggested it as a specific biological test for arsenic.

MAASEN, in 1902, disproved the specificity of GOSIO's reaction by showing that the same fungi were capable of converting salts of tellurium and selenium into volatile odorous compounds. In the case of tellurium the resulting odor was indistinguishable from that derived from arsenic, since both were garlic-like. It was later considered to be diethylarsine oxide and, finally, as a result of very careful studies, CHALLENGER and his co-workers in 1933, gave the formula as $(CH_3)_3As$, or trimethylarsine.

An extensive search of the literature reveals one of GOSIO's significant statements, which is not mentioned elsewhere, and, in the light of modern knowledge on Aspergillosis, assumes a significance probably not understood by that experimenter. Writing in 1901 GOSIO points out that new experiments have convinced him of the following: "Until our present knowledge on *Penicillium brevicaule,* we would consider it as a small cell which, living in contact with a fixed compound of arsenic, produces a toxic gas. But the new research has convinced me that the hyphomycete in question is at times capable of manifesting some direct pathogenic properties, that is to say, to kill the host which contains it as a parasite in the determined organs." Then he states that rabbits shut up in a chamber with an abundance of mycelial growth and spores of *P. brevicaule* die without manifest cause. An autopsy shows a dense mycelium in the lungs which gives cultural characteristics of *P. brevicaule* and reacts on arsenic.

The year 1815 not only saw the first mention of the danger of arsenical wallpapers, but also the year when the first description of fungus infections in animals was made by MAYER. OBICI stated that BENNETT, in 1842, described the first human case of aspergillosis, but it is revealed by CASTELLANI that even into the '50's and '60's many physicians and textbook authors scoffed at the thought of fungi causing diseased conditions.

Aspergillus is found in the skin, eye, ear, and hair. Internally it develops in the nose, bronchi, and lungs with ramifications extending into the liver, kidneys, and spleen. WAHL and ERICKSON give the following as the outstanding symptoms for acute aspergillosis: pleurisy, cough, fever, chills and night sweats, dyspnoea, hemoptysis, fatigue, anorexia, and loss of weight.

In a book, "The Aspergilli," Dr. CHARLES THOM devotes a full chapter to the subject of "Aspergilli and Animal Disease." The same author, in a joint paper with KENNETH B. PAPER entitled "The Arsenic Fungi of Gosio," names the moulds found in his laboratory to be capable of producing the garlic-like Gosio-gas. From these two sources it may be seen that one can obtain "traces of arsenical gas from *A. fumigatus*," and that as an agent of disease, *A. fumigatus* is constantly encountered as a cause of lung lesions in birds, occasionally in man and other mammals, and has been reported occasionally from the human ear, but not definitely shown in skin lesions.

According to THOM and RAPER the odor is likewise produced actively by *A. sydowi* and *A. fischeri*. While the first of these is not reported to be pathogenic, the second is "apparently identical" with *A. fumigatus* and as such may well be covered by the pathogenicity of the latter. Traces of gas are reported from "one of the *A. glaucus* group, and two of the *A. ochraceous* group." Both of these may be found in the list of pathogenic fungi.

Among forms other than the *Aspergilli*, THOM and RAPER obtained a "feeble reaction" from *Penicillium duclauxi*, and "ten strains or species of *Scopulariopsis* (The P. brevicaule series of older authors) were all active gas producers." The latter organism is reported to be pathogenic by GOSIO. Thus five out of the seven moulds which have the power to form volatile arsenicals are also reported to have pathogenic properties. The odor is also formed by some soil fungi which are not completely identified, and the pathogenicity of which is not known.

It would appear advisable to regard the historical "cases of arsenic poisoning from wallpaper" as being due, at least in part, to aspergillosis, penicilliosis or some other causes connected with the damp unhealthy habitats common to most of these cases.

WEGHMAN (1939) reported on mold infection of the *stomach* and the *oesophagus*. At the autopsy of a 65 year old white man who died of sepsis consecutive to a cellulitis of the fifth finger, one found peculiar stomach ulcers. They never showed up during lifetime. The microscopic examination showed that these ulcers have been caused by molds. As possible causative agent *Aspergillus flavescens* was established. The general predisposing factor was seen in the pyogenic infection of long duration, the local predisposing factor probably in hemorrhagic erosions. A specific feature of this observation was the finding of ulcers in the oesophagus caused by molds, not reported on before.

NEGRONI and NEGRI (1939) investigated the soil at Buenos Aires for the flora of *Aspergillus*. 25 compound samples of soil were studied in Buenos Aires to ascertain its *Aspergillus* flora. The majority of the samples were taken from gardens.

The compound samples consisted of a mixture of 2—3 samples taken at the same place with a sampling tube after scraping away with a sterile spatula the grass and the first 1 cm. of soil. The samples were placed in sterile bottles and sowed as quickly as it was possible. One cc. of dilutions of 1/5,000 and 1/10,000 were sowed in acid (pH 5) soil extract and Czapek solid media. Isolation of the colonies were performed after 7—15 days of incubation at 28—30° C.

Nearly half of the soil samples cultivated did not contain *Aspergillus*.

The isolated species of *Aspergillus* were the following in order to their frequency: *Aspergillus niger* group (including *Asp. pseudo citricus* Mosseray, 1934) 5 strains.

Aspergillus terreus: 4 strains.
,, *fumigatus*: 3 strains.
,, *sulphureus group*: 2 strains.
,, *flavus-oryzae*: 2 strains.
,, *nidulans*: 1 strain.

DE ALMEIDA and LACAZ (1939) has reported on the first case of *Acromia Parasitaria* of *Jeanselme* observed in *Sao Paulo.* The causative fungus was determined as *Hormodendrum Fontoynonti.*

DE ALMEIDA (1939) isolated an *Aspergillus* of the group *Ochraceus* from the sputum of an individual suffering from lung affliction. The sputum was constantly negative for acidfast bacilli. The possible pathogenicity of this fungus is discussed. Mycologically it was interesting that this fungus formed sclerotia in the culture.

LINK (1939) described a case of *Fatal Meningitis Aspergillina* in man. This was a true, acute, metastaticembolic *Aspergillus* mycosis of the leptomeninges in a 19 year old man, probably the first case ever observed. The primary focus of the mycosis has probably been in a circumscribed *Pneumomycosis Aspergillina* of the left lung. Due to the development of numerous, strong mycelia in the leptomeninges a suppurative, mainly basal, leptomeningitis set in leading to death within five days. There was no mixed infection. As the only sign of a beginning destructive effect of the fungi on the tissues circumscribed necrosis in the wall of the vessels of the pia mater could be established. The suppuration is conspicuously the first reaction of the tissues against the fungi and the necrosis of the wall of the vessels followed later. Mycoses caused by common molds in man led up to now to chronic inflammatory processes in the organs. The oxygen carried in the vessels of the pia mater is seemingly enough for the development of numerous, strong mycelial elements. Organs of fructification of the fungus has not been observed.

FELDERMAN (1940) discussed infections with *Aspergillus Niger.* The observations have been based on two cases. In both instances the patients complained of pain accompanied by intolerable itching of the organs involved, nervous irritability, insomnia and headache.

In the first patient on washing the canals of the ears, a greenish black wax was delivered and at the end one could see a light greenish corkscrew form, which led to the suspicion of a fungous mass. The washings were immediately referred to the laboratory for study and the report identified the organism as *Aspergillus Niger.*

The second patient showed a "membrane" in the interior of the nose which when removed caused a bleeding surface. The "membrane" re-formed in about 6 to 8 hours. One of the several physicians consulted, made a diagnosis of "*athlete's foot of the nose.*" The membrane in the interior of the nose was thick and of a distinct pinkish hue, obliterating the nasal passages. The culture showed gram-positive diplococci, *Staphylococcus aureus, flavus,* and *B. coli.* The culture yielded also a fungus of the "*Aspergillus* type."

The author found it instructive to consider briefly the fungi responsible for this disease. They include *Absidia ramosa* (Jakowski, 1888), *Aspergillus Siebenmann* (1883), *Aspergillus described* by Macfie, *Penicillium minimum, Castellania copelli, Mycoderma pararogosum, Candida bethalieniss, Pseudomonilia allesandrina.*

He concludes that the automobile, the motor trailer and the aeroplane are the chief carriers of mycotic infections.

R. Fungus infections in animals

Fungus infections in *animals,* wild or domestic, have a definite interest also for human pathology. Firstly, because animals may be the hosts for certain pathogenic fungi for humans; secondly, they may be carriers and/or reservoirs, spreading pathogenic fungi among free living animals. or, by contact, transmitting the pathogenic agents to humans. They have also great economic importance in modern fur animal farms where the finest pelts can be ruined by a fungus infection, and in turn, it can be transmitted to the attendants. It has its pathologic and economic significance also in cattle, horses, in human pets, like dogs and cats. Besides, by observation of fungus diseases in animals our knowledge in comparative pathology may be usefully enlarged.

Mangrulkar (1938) reported on *Actinomycosis* and *actinobacillosis* in animals in *India*. As evidence of the occurrence of this fungus disease, case reports in cattle, water buffalo and dogs have been presented.

Sartory et al. (1939) reported on a case of generalized *Septicemia* due to an actinomyces of the type of *A. Bovis Harz* in a young calf. The portal of entrance of the infecting fungus was probably the navel. A. bovis was shown from the kidneys, liver, lungs, and heart blood. They underscore the danger of eating the meat or internal organs of infected animals, because the extent of the infection can not always be with security determined.

Leue (1939) described a rare case of chronic *Actinomycosis* of the tongue in the swine. The tongue weighed 1030 gms. Its cut surface showed a grayish-white, severely edematous tissue in which yellowish-gray, lentil size nodules were embedded. The nodules showed the typical architecture of the actinomycotic granulation tissue.

Lange (1939) also observed a similar case of *Actinomycosis* of the tongue in the swine. The tongue was 60 cm. long and weighed 3 kg. The tissue was hard and it showed on its cut surface typical actinomycotic granules. Since the granules did not reveal in their center a mash of mycelia, it was presumed that the infection was caused by the *Actinobacillus lignieresi.*

Brion (1939) described *Actinomycosis* in dog and cat. The actinomycosis of the skin may coincide in *Carnivores* with involvement of the serosa, bone, central nervous system and other organs. The most frequent form of the disease is the actinomycosis of the skin, then, that of the serous membranes. Several cases of this infection in cats and dogs were observed and worked up clinically, pathologically and bacteriologically. The etiological agent in these cases was an organism nearly identical with the *Actinomyces Israeli*, yet, it showed some specific features.

S. Varia

Carter et al. (1939) investigated *Vulvovaginal mycoses in pregnancy with the relation of symptoms to genera and species of fungi*. Cultures of yeastlike fungi from the vaginae and vulvae of 200 pregnant women were positive in eighty-six (43 per cent) of the patients. These fungi belonged to the genera *Monilia*, *Saccharomyces* or *Cryptococcus*. Sixty-six (33 per cent) of the patients showed positive vaginal cultures; twenty (10 per cent) showed positive labial cultures with negative vaginal cultures.

A total of 151 strains of yeastlike fungi were classified according to the method previously described by Jones and Martin. Each strain identified as *M. albicans* was pathogenic for rabbits. Each strain identified as *M. stellatoidea* was non-pathogenic for rabbits.

In patients in whom the fungi were identified as belonging to the genera *Saccharomyces* or *Cryptococcus* no symptoms referable to a mycotic infection were described. Symptoms in patients with positive cultures for yeast-like fungi belonging to the genus *Monilia* were found only in those in whom the three species, *albicans*, *stellatoidea* and (candida) *tropicalis* were isolated. Although the incidence of trichomonads was higher in the patients from whom

M. stellatoidea was cultured, more patients complained of pruritus in the group from which *M. albicans* was cultured.

Intradermal skin test with antigens from the five species of *Monilia* showed no correlation between positive cultures and positive intradermal tests. The incidence of *Monilia* was higher in the group with negative skin tests.

The presence or absence of agglutinins in the patient's serum could not be used as an index of vaginal or labial infection with *Monilia.*

Vaginal smear typing showed that *Monilia* were found more frequently in patients with type 2 smears; that trichomonads were found more frequently in patients with type 3 smears.

Additional work must be done on the mycologic flora of the vulva and vagina in normal patients and in patients with mycotic vulvovaginitis before we may understand the relative pathogenicity of the different genera and species of yeastlike fungi and may evaluate methods of diagnosis and treatment.

Pratt (1939) investigated the *mold spore content of the air in Boston.* Following the method of Feinberg, Petri dishes containing Sabouraud's medium were exposed to the outside air for a half hour each day for fourteen months. *Alternaria, Hormodendrum,* and nonsporebearing molds were found to increase in quantity gradually during the spring months, to increase rapidly during July, August, and September, and to taper off during October and November. *Aspergillus, Penicillium,* and other air-borne molds failed to display a seasonal incidence.

Twenty-five per cent of 177 children suffering from asthma and hay fever reacted strongly to Alternaria extracts when tested intracutaneously. A much smaller number reacted to other molds.

Reagins to *Alternaria* could easily be demonstrated in the sera of 92 per cent of 26 children chosen at random from among those giving strong skin reactions to *Alternaria.* Reagins were less frequently present to other molds.

The seasonal incidence of symptoms among those patients giving strong skin tests to molds corresponds closely to the seasonal variations of mold spores in the outdoor air when the effect of coexisting pollen sensitivity is taken into account.

During 1937, 34 patients were treated with mold extracts. Twenty-three of these also received pollen therapy. Of the 11 receiving only mold treatment, 9 obtained marked relief, and only 2 failed to benefit.

Lampa (1940) reported on *Piedra* in *Batavia.* Piedra with black nodules, caused by piedraia, is frequently met in the native population of Batavia; 120 men and 66 women were examined out of whom 40 and 3 were infected, viz. 33 and 5 %. On the other hand piedra with less colored or white nodules, caused by trichosporum, seems very rare; only one case was seen.

No evidence was found of a direct transmission, but a high incidence was observed in men who take their baths in public waters and the same was true for the use of a certain hair-oil, locally made in a Chinese factory. Whether these co-incidences are real and significant in connection with the cause or the manifestation of the disease is yet to be decided.

Waldbott et al. (1940) investigated the *Fungi's relation to respiratory allergy* in Southern Michigan.

After intensive preliminary studies concerning the growth and exposure of plates the authors decided to employ the following method: Sabouraud plates were exposed for thirty minutes daily on top of a four-story building in the center of Detroit for a period of one year. The number of colonies of each genus was counted, identified, and entered on a graph. Realizing the variations to which the results would be subject, the authors attempted to establish as many controls as possible. For instance, plates were exposed simultaneously under the same experimental conditions, throughout the whole duration of the survey. Other controls included simultaneous exposures at four building of different heights in Detroit. The authors also selected 23 different habitats for fungi throughout the city, namely such places as a bakery, butcher shop, pig pen, cow stable, chicken coop, library room, etc., where plates were exposed simultaneously. These controls, it is true, showed certain individual differences in the fungus flora at the different stations; but on the whole a definite pattern of distribution for certain fungi was recognized which tended to tally in all these exposures on certain days and at certain seasons. Precipitation and low barometric pressure tended to free the air temporarily from fungus spores. Inland winds, as well as high barometric pressure, increased the count. Continued freezing and subfreezing weather inhibited propagation of spores.

Only a few fungi showed seasonal appearances, while the majority were present practically throughout the whole year. Among the latter, *Penicillium* showed a marked predominance, some of the counts reaching 138 colonies during the half-hour exposure. Two other forms which the authors consider rather significant clinically are yeasts and *Torula*. The highest counts of yeast and Torula were obtained at the time when relatively few spores of other genera were present in the air, namely, from December to March. *Aspergillus*, which some believe to be very abundant, gave relatively low counts throughout the period of the survey.

Penicillium, yeasts, and *Torula* were most prevalent among the perennial fungi. *Alternaria* and *Monilia* were present throughout the year, but showed seasonal predominance, namely, from July to November, and May to November, respectively. Among fungi which appeared to be seasonal, but which gave only low counts at any time, were *Hormodendrum*, *Macrospora*, *Neurospora*, *Trichothecium*, and *Trichoderma*.

NEGRONI and FISCHER (1940) investigated the *mycologic flora* (*eumycetes*) *of the fecal material*. They have isolated 86 Eumycetes from the feces of 50 patients examined at random from Dr. E. FINOCHIETTO's surgical service in Buenos Aires.

34 of these fungi were classified as *Candida being C. Krusei* the most frequently isolated. The remaining species were the following. 3C. parakrusei, 7 *C. albicans*, 4 *C. zeylanoides*, 3 *C. tropicalis*, and 2 *C. Chalmersi*. The authors describe a new variety of the last fungus: *C. Chalmersi* var. velosa.

They have cultivated 14 strains of the genus *Geotrichum*: 12 *G. candidum*, 1 G. *rotundatum* and a new species *Geotrichum Redaelli*, *Negroni* and *Fischer*.

5 species of *Rhodotorula* were also found; 2 R. *mucilaginosa* and 3 *R. mucilaginosa* var. Carbonei.

As *Saccharomycetaceae* the authors have studied 2 *Saccharomyces Chodati*, 1 *Sacch. Chodati* var. Lodderi, *Negroni* and *Fischer*, (new variety), 3 *Sacch. heterogenicus* and a new variety of *Pichia*: *P. farinosa* var. galerus.

They have classified 7 species of *Aspergillus* (3 *A. glaucus*, 2 *A. niger*, 1 *A. fumigatus*, and 1 *A. versicolor*) and 14 *Penicillium* distributed in the following species: 7 *P. roqueforti*, 2 *P. casei*, 2 *p. cyclopium*, 1 *P. cyaneo-fulvum*, 1 *P. crustosum* and 1 *P. fellutanum*.

Finally the authors have isolated 5 species of *Mucor*: *M. varians*, *M. abundans*, *M. griseo-lilacinus*, *M. racemosus* and *M. spinosus*.

PENNINGTON (1940) investigated *the incidence of air-borne molds and of skin sensitivity to molds*.

The skin tests with 52 mold extracts performed on 526 presumably allergic patients suffering from hay fever and asthma yielded a very large number of positive skin reactions. In this connection it should be remembered that this group, unlike similar large groups reported in the literature, is made up entirely of patients suffering from hay fever and asthma, all of whom were allergic. Thus 438, or 86.5 per cent of the patients tested to either pollen or dust or both, reacted positively. By way of comparison, only 52 per cent of patients in the LAMSON and ROGERS group reacted to other allergens.

The author was impressed by the difference in reactivity to different species of the same genus and even to different varieties or strains of the same species. In order to determine if there were completely crossed reactions between all or any species or varieties of one genus, he chose reactions to 9 extracts of the genus *Aspergillus* for more detailled analysis. He selected *Aspergillus* for this study because four species of this genus had also been studied by LAMSON and ROGERS. They found no evidence of completely crossed reactions between any of the four species of this genus studied by them. The author has had a similar experience. He observed no completely crossed reactions between the 5 species selected by him, nor between the 2 strains of one species (*Aspergillus fumigatus*) and the 4 strains of another species (*Aspergillus niger*). It appeared, therefore, that there may be a genus specific as well as a species specific and perhaps a variety specific factor, at least in the species and varieties which the author employed in this study.

The importance of the question of completely crossed reactions becomes obvious when it is recalled that the demonstration of their presence would mean that one potent extract of each genus would be sufficient for testing. If reactions between the species of a genus or even between the variaties of a species were completely crossed, it would greatly simplify testing for moulds without the danger of overlooking many instances of mold sensitiveness.

Positive skin reactions were obtained in most patients to more than one mold, indicating multiple skin sensitivity. Few patients reacted to molds only, all except two mold sensitive patients being sensitive to other allergens.

An effort has been made to correlate skin sensitivity to molds with clinical sensitivity. Investigations along this line are in progress and will be made the subject of further study. Many of the patients exhibiting two-plus and one-plus skin tests to molds have been shown to be clinically sensitive. Many have been treated to molds with improvement in the clinical symptoms. A large percentage of allergic patients are skin sensitive to air-borne molds.

A large variety of mold spores should be included in the list of possible mold allergens. More investigative clinical work must be done and more widely scattered mold surveys must be made before it can be determined what mold genera and species may be antigenic.

No evidence of completely crossed reactions was found among the species of any mold genus, or even among the strains and vatieties of a species.

It is not always possible to evaluate the factor of mold allergy in the production of allergic symptoms; skin sensitivity to molds is usually multiple and coexists with sensitivity to food and other inhalant allergens.

Air-borne mold spores must take their place along with foods, pollens and other inhalants as an important cause of allergic disease.

WALDBOTT and BLAIR (1940) evaluated the *importance of fungi in respiratory allergy*. The role played by fungi in respiratory allergy (asthma and allergic nasal disease) was investigated by an atmospheric survey of fungi and a clinical study of 841 patients with upper respiratory allergy was made. This study covered intradermal skin tests, fungus cultures, and microscopic examinations of nasal and bronchial secretions, a follow-up of the dates of the patients symptoms by means of "symptom calendars." and fungus surveys in the patients' homes.

Skin reactions to one or more fungi were positive in 69 per cent of the 841 allergic patients. Fewer positive reactions were obtained to fungi than the pollens and epidermals but about as many as to foods. The strongest positive reactions to fungi occurred in patients who also reacted strongly positive to other antigens.

In the cultures from nasal and bronchial secretions, *Penicillium, Alternaria, Monilia,* and yeast prevailed. During October and November fungi grew more abundantly from the respiratory secretions than at any other period.

Plates exposed in patients' homes revealed no characteristic habitats of fungi in any single case.

The follow-up of the dates of symptoms indicates that respiratory allergy due to one single fungus is very rare. More often fungi are complicating factors in multiple sensitive patients with asthma and vasomotor rhinitis. During the summer months the most common offenders are smut, *Alternaria,* and rust. As such they should be given special consideration in the treatment of hay fever. *Penicillium* which was by far the most common fungus found in the atmosphere, homes and the mucous secretions, was of relatively minor significance clinically.

Some of our cases were found to merely simulate asthma and vasomotor-rhinitis. They represented true fungus infections. The diagnostic criteria in these cases are outlined.

ANDERSON (1941) reported on *fungous infections of the brain.*

Several collective studies are on record, one of the most important of which is the review by BOYD and CRUTCHFIELD in 1921. In 1928 Jacobson reported 4 cases, in 1 of which there was cerebral involvement, and set the number of recorded cases at that time at 92. In 1936 the California state department of health reported 450 cases, most of which had occurred in California, with 224 deaths. The number of cerebral complications in these cases was not mentioned, but Beck, in a review of 286 cases occurring in California up to 1931, had found intracranial lesions in 18. COURVILLE and ABBOTT, who in 1938 reviewed the cases in the Los Angeles County Hospital, observed lesions of the nervous system in about 25 per cent of those in which autopsy was performed.

DUCKETT and FREDEEN, in 1936, reported a case of hydrocephalus associated with convulsions in a child 5 years of age. The ependymal linings of the ventricles were affected, but no mention was made of a discrete granuloma. STORTS in 1939 reported a case of coccidioidal granuloma simulating brain tumor in a child 4 years of age. He stated that this case brought the number of such conditions occurring in children under 5 years of age to 22. Four cases of fungous infection of the brain are reported, in each of which the condition was due to a different cause (case 1, coccidiosis; case 2, saccharomycosis; case 3, actinomycosis; case 4, infection with a mycelium-producing organism).

In 2 cases the lesions were abscesses of the brain, and in 2, encephalomeningitis.

In all 4 cases the diagnosis was verified by microscopic examination, and in 3 it was confirmed by autopsy. In the fourth case the culture yielded bacteria.

The preoperative diagnosis in 2 cases was tumor of the brain.

All 4 cases terminated in death.

BEARSE reported on *mycotic infections of the stomach.* Fungi may cause a gastritis, ulceration and even go on to perforation.

There are no characteristic symptoms. The complaints are similar to those in functional disturbances of the stomach or in peptic ulcer.

The diagnosis should be thought of when pathogenic fungi are found in the vomitus. The suspicion should be strengthened when these fungi are found in uncontaminated gastric contents and can also be cultured in appropriate media. Should the inoculation of this culture into a rabbit give a typical reaction and be corroborated by positive blood cultures, intradermal tests and blood agglutinations, a diagnosis of a mycotic infection is warranted.

The more frequent use of the gastroscope together with increasing skill in recognizing typical mycotic gastritis or ulceration should help to make an early diagnosis possible.

No reliable prognosis can be given owing to a lack of cases on which to base a definite opinion. There were no complications that could be attributed to the fungi themselves in those cases where operation was performed. If the lesion is due to the ray fungus, the prognosis must be definitely guarded due to the possibility that metastasis to the liver may already be present.

In uncomplicated cases, iodides to the point of tolerance should be administered. If there are actinomycotic sinuses, then X-ray treatment should also be used.

For complications such as perforation or repeated hemorrhages, operation is necessary.

PENNINGTON (1941) has studied the *clinical sensitivity to air-borne molds.* In a previous study the incidence of positive skin tests to air-borne molds in a group of 526 patients was reported. The present communication is concerned with attempts to discover and correlate clinical manifestations of sensitivity to molds with skin sensitivity in patients selected from the above group.

All of the patients in the original group had asthma or hay fever, and with two exceptions all reacted to other allergens. Eighty-five per cent of them developed positive skin reactions to one or more of fifty-two mold extracts. These patients were tested and treated over a period of three years, during which concurrent studies of the atmospheric molds were made. They were tested and treated with molds most commonly found in the air, several strains of each most frequently encountered being employed. Thus, patients were tested with five strains of *Alternaria,* six of *Hormodendrum,* four of *Aspergillus niger,* two of *Aspergillus fumigatus,* and three other strains of *Aspergilli.*

It was not assumed that 85 per cent of the 526 patients were clinically sensitive to molds, nor was it assumed that patients who gave violent positive skin tests to molds were clinically sensitive. Patents exhibiting marked positive reactions to several or all strains of one species or genus, however, were viewed with more suspicion than were those reacting to only one strain of a species or genus.

Skin sensitivity to one or more molds probably means no more than skin sensitivity to one or more foods. As is well known, skin reactions to foods and other allergens are elicited in a large percentage of people tested. Thus, RACKEMANN and SIMON obtained reactions in thirty of sixty normal non-allergic persons. Nine common allergens were used. In our experience reactions to one or more mold extracts failed to occur in only 15 per cent of allergic persons. Skin reactions at best are only clues in relation to the existence of clinical sensitivity. Sixty-seven of a group of 526 patients gave histories strongly suggestive of mold sensitivity.

REAGINS to molds were demonstrated by passive transfer in thirteen of eighteen patients skin-sensitive to molds. The size and intensity of the direct skin test was indicative but not diagnostic of the presence of reagins.

Thirty-three of 526 patients, or 7.3 per cent of the total number, developed systemic reactions to mold injections.

Provocative tests were made on sixty-one patients who were skin sensitive to mold, and twenty-two, or 36 per cent, of these gave positive provocative reactions, thus demonstrating the presence of clinical sensitivity.

Mold sensitivity is not uncommon and must be taken into account in any complete study of the allergic state.

WADE and MATTHEWS (1940) reported on a doubtful cutaneous Mucor Infection of the face. A 17 year old white youth showed on the right cheek two lesions. The larger lesion was approximately round and 2 cm. in diameter; it presented an appearance of dark red jelly-like papillomatous tissue from which exuded a small quantity of serosanguineous fluid. The smallest lesion, the size of a split pea, was likewise raised, and reddish, but showed a smooth surface with no "weeping". There was no induration, redness, or tenderness about either lesion. This condition was of two weeks' duration. Iodide administration (K I by mouth, Na I intravenously) brought the lesions to complete healing without scarring. Surface tissue and the exudate were cultured. The cultures yielded a *Mucor* species which was not examined further mycologically.

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